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Counsel for Plaintiffs,

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

SAN FRANCISCO DIVISION

THERANOS, INC. and ELIZABETH HOLMES,

Plaintiffs,

v.

FUISZ TECHNOLOGIES, LTD., JOHN R. FUISZ, RICHARD C. FUISZ, and JOSEPH M. FUISZ,

Defendants.

CASE NO.:

COMPLAINT AND JURY DEMAND

COMPLAINT

Plaintiffs Theranos Inc. ("Theranos") and Elizabeth Holmes, by and through their undersigned counsel, state, with knowledge of their own acts and status and acts taking place in their presence, and upon information and belief as

to all other matters, as follows:

Nature of the Action

1. This is an action for damages and equitable relief arising from the tortious and otherwise wrongful conduct of Defendants Fuisz Technologies, Ltd., John R. Fuisz, Richard C. Fuisz, and Joseph M. Fuisz in, among other things, misappropriating and using Plaintiffs' confidential information, including proprietary data and intellectual property ("Confidential Information").

The Parties

2. Theranos is a corporation organized under the laws of the state of Delaware with its principal place of business in Palo Alto, California. Theranos is a healthcare systems company that develops groundbreaking technologies used in the collection, analysis, and communication of health information. Based on its highly valuable and highly praised inventions and intellectual property, Theranos has created integrated systems, methods, and processes for real-time testing, decision making, and individualized therapy that deliver faster, more accurate, and less expensive health care to physicians, patients, and consumers.

3. Elizabeth Holmes is a resident of Palo Alto, California. Ms. Holmes founded Theranos in 2003 and is a named inventor on several patents, including patents covering bodily fluid analyzers and systems. Ms. Holmes is currently the CEO of Theranos.

4. Defendant Fuisz Technologies, Ltd. ("Fuisz Technologies") is a corporation formed under the laws of the state of Delaware and has its principal place of business in the state of Virginia. According to its website, Fuisz Technologies is a family-held company and specializes in drug delivery systems.

5. Defendant John R. Fuisz is a resident of Washington, D.C. John Fuisz is a lawyer and was formerly a partner at a multistate law firm with offices in California and Washington, D.C. ("John Fuisz's Law Firm"). John Fuisz

joined John Fuisz's Law Firm's Washington, D.C. office in 1999 and mainly practiced patent litigation during the relevant time period. He also was a member of the board of directors of the family-owned Fuisz Technologies.

6. Defendant Richard C. Fuisz is a resident of the state of California. Richard Fuisz is the father of John Fuisz. Richard Fuisz is described as a founder of Fuisz Technologies in Fuisz Technologies press releases.

7. Defendant Joseph M. Fuisz (together with John Fuisz and Richard Fuisz, the "Individual Defendants") is a resident of the state of Florida. Joseph Fuisz is the brother of John Fuisz. Joseph Fuisz is described as a managing member of Fuisz Technologies in Fuisz Technologies press releases.

Jurisdiction and Venue

8. Jurisdiction is proper in this Court pursuant to 28 U.S.C. §§ 1331 1338, and 1367.

9. This Court has personal jurisdiction over Defendant John Fuisz, including because he wrongfully disclosed the confidential and proprietary information of a California-based company and inventors known to reside in California, and conspired to misappropriate and use, to Theranos' and the inventors' detriment, such confidential information, and thus purposefully directed his conduct toward California. Moreover, during the relevant time period, John Fuisz's Law Firm had offices, partners, and employees within California and conducted business within California.

10. This Court has personal jurisdiction over Defendants Fuisz Technologies, Richard Fuisz, and Joseph Fuisz, including because they wrongfully acquired the information and business of a California-based company and inventors known to reside in California, and thus purposefully directed their conduct toward California. Defendants Richard and Joseph Fuisz also conspired with John Fuisz to wrongfully acquire, and did wrongfully acquire, the confidential and proprietary information of a California-based company and

inventors known to reside in California, and thus purposefully directed their conduct toward California.

11. Venue is proper under 28 U.S.C. § 1391 because a substantial part of the events giving rise to the claims asserted herein took place in this District.

Factual Allegations

A. Ms. Holmes, Founder and CEO of Theranos, Is an Inventor of Revolutionary Health Care Technology.

12. Ms. Holmes conceived the technology upon which Theranos was founded while a chemical and electrical engineering student at Stanford University. Determined to develop a means of providing accurate, cost-effective, real-time medical data to medical professionals and patients at the time of diagnosis and treatment, beginning in 2003 Ms. Holmes devoted herself to inventing the technology, methods, and systems to realize that vision. Accordingly, she dropped out of Stanford University at the age of 19 to found Theranos and invested the funds she had reserved for her college education into achieving her goal.

13. The technology, methods, and systems Ms. Holmes envisioned, and has since worked to create, promise to revolutionize healthcare by making it possible to analyze a patient's blood and wirelessly transmit that analysis to a secure database, where additional analyses can take place and from which the patient's physician can access the results and data. With one small prick of a finger, patients can transmit real-time medical data and receive real-time feedback from their health care provider.

14. Plaintiffs' groundbreaking discoveries and inventions comprise significant and valuable advances not only with respect to the technology available for the healthcare, biotechnical, and pharmaceutical industries, but also,

most importantly, with respect to improving medical research, point-of-care treatment, and the quality and cost of healthcare.

B. Plaintiffs Retained John Fuisz's Law Firm to Prosecute Patents.

15. In or about August 2003, Plaintiffs retained John Fuisz's Law Firm to provide legal services to Plaintiffs in prosecuting patent applications covering Plaintiffs' inventions. Between 2003 and approximately early 2006, John Fuisz's Law Firm represented Plaintiffs in the preparation, filing, and prosecution of their domestic and international patent applications, and continued to represent Plaintiffs through October 2008.

16. During this representation, John Fuisz's Law Firm provided legal services to Plaintiffs, including services related to the preparation, filing, and prosecution of Plaintiffs' patent applications, mainly out of its office located in Washington, D.C., and in so doing reviewed the information and materials that Plaintiffs sent from California in order to prosecute such applications and communicated with Plaintiffs in California.

17. During John Fuisz's Law Firm's representation of Plaintiffs, its patent attorneys were divided into various groups, including the IP Litigation group and the Patent Prosecution group.

18. John Fuisz's Law Firm permitted attorneys in the IP Litigation and the Patent Prosecution groups to access the same set of documents and data, including to search and access electronic documents, and making available a central file room or rooms that included patent-related hard copy materials.

19. All attorneys, in both the IP Litigation group and the Patent Prosecution group, had unfettered access to confidential and proprietary information included in Plaintiffs' patent application files.

C. John Fuisz's Law Firm Drafted and Filed Provisional Patent Applications with the U.S. Patent and Trademark Office on Plaintiffs' Behalf.

20. Between about August 2003 and early 2006, the work Plaintiffs entrusted to John Fuisz's Law Firm including preparing and filing various provisional patent applications on their behalf.

21. In 2005, as part of its work for Plaintiffs, John Fuisz's Law Firm drafted and filed several provisional patent applications, including U.S. Provisional Patent Application No. 60/678,801, filed on May 9, 2005; U.S. Provisional Patent Application No. 60/705,489, filed on Aug. 5, 2005; U.S. Provisional Patent Application No. 60/717,192, filed on Sep. 16, 2005; and U.S. Provisional Patent Application No. 60/721,097, filed on Sep. 28, 2005 (collectively, the "Theranos Provisionals"). The Theranos Provisionals are attached hereto as Exhibit A, B, C, and D.

22. Provisional patent applications provide an applicant with an early filing date, while allowing the applicant to wait up to a year to file a non-provisional patent application with specific claims. The contents of provisional patent applications often contain confidential and proprietary information that ultimately will support the inventor's effort to demonstrate to the United States Patent and Trademark Office ("PTO") that his or her application includes patentable (*e.g.* novel) subject matter.

23. The Theranos Provisionals included confidential and proprietary information.

24. In or about early 2006, another law firm took over the preparation, filing, and prosecution of subsequent patent applications.

25. Plaintiffs filed several patent applications on March 24, 2006 (the "March 24, 2006 Applications") that claim priority to the Theranos Provisionals.

26. Prior to the publication of the March 24, 2006 Applications in November 2007, the research, technology, and materials disclosed in the

Theranos Provisionals were closely guarded and not publicly available. Until publication of the March 24, 2006 Applications, the Theranos Provisionals and the March 24, 2006 Applications themselves were also confidential.

27. The March 24, 2006 Applications were not published until November 16, 2006, at the earliest.

D. Defendants Wrongfully Acquired Plaintiffs' Confidential Information and Used It to File Their Own Provisional Patent Application.

28. Defendants Richard, John, and Joseph Fuisz conspired and agreed to wrongfully misappropriate the valuable confidential and proprietary information that Plaintiffs had entrusted to John Fuisz's Law Firm, including both the general knowledge of Plaintiffs' work and plans and specific information set forth in the Theranos Provisionals, and in other materials submitted in confidence by Plaintiffs to John Fuisz's Law Firm.

29. In April 2006, Richard and Joseph Fuisz filed a provisional patent application with the serial number 60/794,117 (the "Fuisz Provisional Patent Application"). The Fuisz Provisional Patent Application is attached hereto as Exhibit E.

30. The Fuisz Provisional Patent Application ultimately matured into U.S. Patent No. 7,824,612 (the "'612 Patent").

31. Pursuant to Defendants' conspiracy, John Fuisz misappropriated the confidential information, data, and materials of Plaintiffs, including the Theranos Provisionals and related materials and information, and furnished them to his father and brother, who used the information to file the Fuisz Provisional Patent Application in April 2006.

32. Neither the Theranos Provisionals nor the March 24, 2006 Applications were published—and, therefore, were not publicly available—until November 16, 2006, at the earliest.

33. When Richard and Joseph Fuisz filed the Fuisz Provisional Patent Application in April 2006, the Theranos Provisionals had not yet been published. Thus, Richard and Joseph Fuisz should not have had access to the information, data, or materials in the Theranos Provisionals or any other confidential information that Plaintiffs provided to John Fuisz's Law Firm.

34. Nevertheless, the Fuisz Provisional Patent Application included material from the non-public Theranos Provisionals, as well as from other confidential documents and data Plaintiffs provided to John Fuisz's Law Firm in connection with the prosecution of Plaintiffs' patent applications.

35. The concepts and disclosures in the Fuisz Provisional Patent Application closely track those in the Theranos Provisionals.

36. For example, the Fuisz Provisional Patent Application states that the "invention" therein includes "a data reader unit for reading information from a data storage unit, the data storage unit containing stored information concerning a particular drug being or to be taken by the patient."

37. Theranos Provisional Patent Application No. 60/678,801 specifically describes a reader device that "could transmit the results of the analysis to an external database and is also capable of receiving data from such databases." The same Theranos Provisional Patent Application also states that "stored information" could be information from the patient's "previous treatment regiment," or "it could be pharmacogenomic data that are of relevance to a particular patient."

38. The Fuisz Provisional Patent Application also discusses "processing the information concerning the analyte and for sending the processed information to the display, wherein the threshold value is associated with the particular drug being or to be taken by the patient or course or treatment by the patient, the threshold value being one beyond which the display will display an alert."

39. The same concepts are detailed in the Theranos Provisionals. Specifically, these provisional patent applications describe a bodily fluid analyzer that reads at least one value from the data reader, compares the value with data retrieved from a database, and evaluates whether the comparison exceeded a predetermined threshold value.

40. Theranos Provisional Patent Application No. 60/678,801, for example, describes a method through which "data can be compared with information stored in databases."

41. Theranos Provisional Patent Application No. 60/717,192 further expands this concept to make it clear that an "action threshold value" could be used to "determine the optimum therapeutic index for that particular patient or patient class." It further provides that if the "threshold value" were exceeded, "appropriate action" could be taken, such as generating "an alert," including to a healthcare provider.

42. In addition, Theranos Provisional Patent Application No. 60/678,801, for example, explains "One kind of immediate action" that could be taken if a threshold value were exceeded "could be to provide an emergency alert to the patient's healthcare provider."

43. Furthermore, Theranos Provisional Patent Application No. 60/717,192 describes "alerting the physician" among other steps that could be taken if a "threshold value" was exceeded.

44. Theranos Provisional Patent Application No. 60/705,489 further describes displaying information reflective of a comparison of a sensed value with an expected value.

45. Additional similarities can be found between the concepts and information set forth in the Theranos Provisionals and other of Plaintiffs' Confidential Information provided to John Fuisz's Law Firm and other disclosures in the Fuisz Provisional Patent Application.

46. The Fuisz Provisional Patent Application, for example, states that "the data storage unit can be a bar code and the data reader can be a bar code reader."

47. Theranos Provisional Patent Application No. 60/705,489, which predates the Fuisz Provisional Patent Application, describes the concept of using a bar code to read and store information, including where "software allows reading of a barcode on the cartridge" and a "server" can then transmit "a preprogrammed reading based on each cartridge bar code" to a "read-out display."

48. In addition to the Theranos Provisionals, confidential materials Plaintiffs provided to John Fuisz's Law Firm also detail novel concepts that later appeared in the Fuisz Provisional Patent Application. One such concept includes making "a diagnostic and therapeutic system" with the ability "to serve as a much sought-after application for PAN or RFID technologies; capitalize on entry into wireless, microchip markets." The term "RFID" stands for "Radio Frequency Identification Device" and refers to the transmission of information by certain radio wave-based wireless technologies.

49. Correspondingly, the later-filed Fuisz Provisional Patent Application states that "the data storage unit can be a radio frequency receiver identification tag and the data reader a radio frequency receiver," which is an application of "RFID technologies."

E. Plaintiffs Discovered the Fuisz Provisional Application and That John Fuisz Was a Partner in the IP Group of John Fuisz's Law Firm.

50. Shortly before October 28, 2008, Ms. Holmes was informed that defendant John Fuisz was a member of the IP Litigation group at John Fuisz's Law Firm.

51. Since Ms. Holmes had also learned that Defendant Richard Fuisz, Defendant John Fuisz's father, was prosecuting a patent application that was

substantially similar to a patent application that she and Theranos were prosecuting, she immediately contacted attorneys at John Fuisz's Law Firm and informed them that the father of a partner in their office had filed an application for a patent claiming priority to the Fuisz Provisional Patent Application, which contained concepts substantially similar to those disclosed in the Theranos Provisionals.

52. Shortly thereafter, John Fuisz's Law Firm's attorneys responded that there was "nothing" they could do to remedy the situation.

53. John Fuisz is no longer employed by John Fuisz's Law Firm.

F. Richard and Joseph Fuisz's Patent Was Issued on November 2, 2010, and the Issuance of that Patent Harmed and Continues to Harm Plaintiffs.

54. On November 2, 2010, the PTO issued the '612 Patent, on which Richard and Joseph Fuisz are identified as inventors. The '612 Patent was based on the Fuisz Provisional Patent Application of April 2006.

55. Richard and Joseph Fuisz assigned or otherwise transferred the '612 Patent to Fuisz Technologies.

56. Fuisz Technologies' website currently lists the '612 Patent among a list of "Fuisz invented United States patents and pending patents."

57. The issuance of the '612 Patent harmed and continues to harm Plaintiffs.

58. The biotechnology field is competitive. A critical factor to a company's success in this field is the protection of its confidential and proprietary information. One of the reasons this factor is critical for a company's success is that research and development of a product requires significant investment, with no guarantee that the investment will later generate a return. Without the protection of confidential and proprietary technology enabling, among other

things, the right to exclude others from manufacturing and monetizing products, a biotechnology company is unlikely to be able to recover its upfront investments.

59. Because the '612 Patent covers technology based on Plaintiffs' Confidential Information, including the Theranos Provisionals, Theranos' ability to enjoy exclusively its confidential and proprietary information, and inventions based thereon, has been significantly damaged.

60. In a press release dated July 2, 2010 concerning the '612 Patent, Fuisz Technologies stated:

> First, FUISZ announced that it has received a notice of issuance from the United States Patent and Trademark Office for patent claims that cover a direct connection between prescribing physicians and blood analysis devices. The invention allows for the health care provider to set the parameters of allowable analyte levels for each patient and enables simple notifications for any variance out of a pre-determined range. Joseph Fuisz, managing member of FUISZ stated, "These patent claims represents [sic] a quantum leap in the use of portable blood analyzers to provide a higher level of care for patients while reducing burdens on health care providers. ***We are in discussions with the blood analyzer industry for the licensing of this technology***."

(Emphasis added).

61. Fuisz Technologies and the Individual Defendants also have announced that they have released or will release products based on the same technology.

62. In a press release dated February 7, 2011, Fuisz Pharma, another one of the Fuisz family companies in which Joseph Fuisz serves as CEO, announced that it has created "a new class of microchip":

> MIAMI, Feb. 7, 2011 /PRNewswire/ -- Based on U.S. Patent 7,824,612 ("Body Fluid Analyzer and System

> including Same and Method for Programming Same"), Fuisz Pharma today announced the use of their patented Technology to create a new class of microchip containing smart tablets that communicate with personal body fluid analyzers.
>
> These tablets wirelessly inform a body fluid analyzer of acceptable analyte values for body fluids, set by the drug company so that the analyzer can provide alerts where the patient's results exceed a threshold value.
>
> Joseph Matus Fuisz, CEO of Fuisz Pharma, states, "We are seeing extraordinarily exciting developments around the use of microchip enabled smart tablets that can wirelessly communicate pertinent information to receiving devices. At the same time, we are seeing further growth in the capabilities of personal body fluid analyzers and a greater appreciation for their use in drug development and personalized medicine. Thus, we see the use of our patent 7,824,612 to enable the value added connection of tablet smart chips together with personal analyzers to convey a broad spectrum of pertinent information. This enhances the function of smart tablets and analyzers alike."

CLAIMS FOR RELIEF

First Claim
Non-Joinder Pursuant to 35 U.S.C. § 256
(Against All Defendants)

63. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 60 as if fully set forth herein.

64. Ms. Holmes is a named co-inventor on one or more patent applications assigned to Theranos, including the Theranos Provisionals.

65. Ms. Holmes conceived certain claimed subject matter in the '612 Patent. Her inventions are present in multiple claims, including at least claims 1, 2, 4, 5, 7, 8, 9, 11, 13-16, 18, and 19, which capture Confidential Information wrongfully acquired by Defendants as discussed in Section D above.

66. Theranos is the owner by assignment from Ms. Holmes of all intellectual property referenced in the preceding two paragraphs.

67. Theranos is the rightful owner of the '612 Patent.

68. Timothy Kemp is a named co-inventor on one or more patent applications assigned to Theranos, including the Theranos Provisionals.

69. Mr. Kemp conceived of certain claimed subject matter in the '612 Patent. His inventions are present in multiple claims, including at least claims 3, 6, 10, 12, and 17, which capture Confidential Information wrongfully acquired by Defendants as discussed in Section D above.

70. Theranos is the owner by assignment from Mr. Kemp of all intellectual property referenced in the preceding two paragraphs.

71. Because of the activities of Defendants discussed above, the labors of Ms. Holmes and Mr. Kemp were conjoined with the efforts of Richard Fuisz and Joseph Fuisz in prosecuting the patent application that became the '612 Patent.

72. Ms. Holmes and Mr. Kemp were not named as inventors on the '612 Patent. Their omission was error, which error arose without deceptive intent on either of their parts.

Second Claim
Misjoinder Pursuant to 35 U.S.C. § 256
(Against All Defendants)

73. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 70 as if fully set forth herein.

74. Neither Richard Fuisz nor Joseph Fuisz, the named inventors on the '612 Patent, made any contribution to the conception of the claimed subject matter of the '612 Patent. Their inclusion as inventors was in error.

Third Claim
Breach of Fiduciary Duties
(Against Defendant John Fuisz)

75. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 72 as if fully set forth herein.

76. John Fuisz's Law Firm owed fiduciary duties to Plaintiffs because it was Plaintiffs' attorney and performed legal services on behalf of Plaintiffs.

77. As a member of that firm, Defendant John Fuisz also owed fiduciary duties to Plaintiffs.

78. Defendant John Fuisz breached his fiduciary duties to Plaintiffs when he misappropriated Confidential Information, including the Theranos Provisionals. John Fuisz further breached his fiduciary duties to Plaintiffs when he furnished Confidential Information to his father, brother, and family-owned Fuisz Technologies that enabled Defendants Richard Fuisz and Joseph Fuisz to obtain the '612 Patent based on such Confidential Information and then to assign or transfer the '612 Patent to Fuisz Technologies.

79. As a direct and proximate cause of Defendant John Fuisz's breach of his fiduciary duties, Plaintiffs have been harmed in an amount to be determined at trial and will continue to be harmed unless appropriate injunctive relief is granted.

Fourth Claim
Aiding and Abetting and Inducing Breach of Fiduciary Duties
(Against All Defendants)

80. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 77 as if fully set forth herein.

81. Defendants Fuisz Technologies, Richard Fuisz, and Joseph Fuisz knew that John Fuisz was a fiduciary to Plaintiffs and owed Plaintiffs fiduciary duties.

82. With such knowledge, Defendants Fuisz Technologies, Richard Fuisz, and Joseph Fuisz nevertheless agreed, encouraged, and assisted John Fuisz in the breaching of his fiduciary duties to Plaintiffs.

83. Pursuant to an agreement and conspiracy with Defendants Fuisz Technologies, Richard Fuisz, and Joseph Fuisz, John Fuisz breached his fiduciary duties to Plaintiffs, and aided, abetted, and induced a breach of John Fuisz's Law Firm's fiduciary duty to Plaintiffs, by obtaining, using, and transmitting Plaintiffs' Confidential Information that enabled Defendants Richard Fuisz and Joseph Fuisz to obtain the '612 Patent based on such information and then to assign or transfer the '612 Patent to Fuisz Technologies.

84. Defendants knew that John Fuisz's Law Firm was a fiduciary to Plaintiffs and owed Plaintiffs fiduciary duties.

85. With such knowledge, Defendants aided, abetted, and induced a breach of John Fuisz's Law Firm's fiduciary duties to Plaintiffs by obtaining, using, and transmitting Plaintiffs' Confidential Information that enabled Defendants Richard Fuisz and Joseph Fuisz to obtain the '612 Patent based on such information and then to assign or transfer the '612 Patent to Fuisz Technologies.

86. As a direct and proximate cause of Defendants' aiding, abetting, and inducing breaches of fiduciary duties, Plaintiffs have been harmed in an amount to be determined at trial and will continue to be harmed until appropriate injunctive relief is granted.

Fifth Claim
Legal Malpractice
(Against Defendant John Fuisz)

87. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 84 as if fully set forth herein.

88. An attorney-client relationship existed between Defendant John Fuisz and Plaintiffs.

89. Because of the attorney-client relationship, John Fuisz had a duty of care to use such skill, prudence, and diligence as a member of the profession commonly possesses.

90. In addition, because of the attorney-client relationship, John Fuisz owed a duty of loyalty to Plaintiffs such that he was obligated not to disclose confidential information entrusted to John Fuisz's Law Firm by Plaintiffs or use any information provided by Plaintiffs for his own direct or indirect personal gains.

91. Defendant John Fuisz breached his duties of care and loyalty to Plaintiffs when he misappropriated Plaintiffs' Confidential Information, and further breached his duties of care and loyalty to Plaintiffs when he furnished Plaintiffs' Confidential Information to his father and brother, who used such confidential files, data, or information to obtain the '612 Patent and then assigned or otherwise transferred the '612 Patent to Fuisz Technologies.

92. As a direct and proximate cause of Defendant John Fuisz's conduct, Plaintiffs have been harmed in an amount to be determined at trial and will continue to be harmed until appropriate injunctive relief is granted.

Sixth Claim
Unjust Enrichment / Restitution
(Against All Defendants)

93. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 90 as if fully set forth herein.

94. The Defendants were enriched by improperly obtaining Plaintiffs' Confidential Information and the '612 Patent at Plaintiffs' expense.

95. As a direct and proximate cause of Defendants' wrongful conduct, Plaintiffs have been harmed in an amount to be determined at trial, and will continue to be harmed until appropriate injunctive relief is granted.

96. Under these circumstances, equity and good conscience would not permit Defendants to retain any ill-gotten gains.

Seventh Claim
Constructive Trust
(Against All Defendants)

97. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 94 as if fully set forth herein.

98. Through the efforts of Ms. Holmes and other inventors, Plaintiffs developed, and therefore became the rightful owners of the Confidential Information.

99. Defendant John Fuisz, through improper means and in violation of Plaintiffs' trust, misappropriated Plaintiffs' Confidential Information, then furnished the Confidential Information to his father, brother, and family-owned Fuisz Technologies, who used the Confidential Information to obtain the '612 Patent.

100. All Defendants conspired to misappropriate and use, through wrongful means and in violation of Plaintiffs' trust, and to Theranos' and other inventors' detriment, such Confidential Information.

101. All Defendants were enriched by improperly misappropriating Plaintiffs' Confidential Information and obtaining the '612 Patent at Plaintiffs' expense.

102. As a direct and proximate cause of Defendants' misconduct, Plaintiffs have been harmed in an amount to be determined at trial and will continue to be harmed unless appropriate injunctive relief is granted.

103. Under these circumstances, equity and good conscience would not permit Defendants to retain any ill-gotten gains. A constructive trust therefore must be preliminarily and permanently imposed upon the '612 Patent and any benefits Defendants have derived from their wrongful conduct and Defendants must be declared constructive or involuntary trustees holding the '612 Patent and any ill-gotten gains for the benefit of Plaintiffs.

Eighth Claim
Unfair Competition / Unfair Business Practice
(Against All Defendants)

104. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 101 as if fully set forth herein.

105. Defendants engaged in unlawful, unethical, or immoral acts or practices when they misappropriated, used, and transmitted Plaintiffs' Confidential Information.

106. As a direct and proximate cause of Defendants' unlawful, unethical, or immoral acts or practices, Plaintiffs have been harmed in an amount to be determined at trial, and will continue to be harmed until appropriate injunctive relief is granted.

107. Plaintiffs could not have reasonably prevented Defendant John Fuisz's unlawful, unethical, or immoral acts or practices or prevented Defendants Richard and Joseph Fuisz from obtaining the '612 Patent.

Ninth Claim
Breach of Contract
(Against John Fuisz)

108. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 105 as if fully set forth herein.

109. Plaintiffs and John Fuisz's Law Firm, through an agreement, course of dealing, and otherwise, entered into a contractual relationship through which John Fuisz's Law Firm provided legal services to Plaintiffs. Pursuant to that agreement and understanding, John Fuisz's Law Firm was obligated to maintain the confidentiality of Plaintiffs' Confidential Information. As a member of that firm, the same contract was binding upon John Fuisz.

110. Defendant John Fuisz breached the contract when he misappropriated Plaintiffs' Confidential Information and furnished such Confidential Information to Defendants Richard Fuisz, Joseph Fuisz, and Fuisz Technologies.

111. John Fuisz breached the agreements with Plaintiffs because he misappropriated Plaintiffs' Confidential Information. As a direct and proximate cause of Defendant John Fuisz's breach of contract, Plaintiffs have been harmed in an amount to be determined at trial and will continue to be harmed until appropriate injunctive relief is granted.

Tenth Claim
Inducing Breach of Contract
(Against Defendants Richard Fuisz, Joseph Fuisz, and Fuisz Technologies)

112. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 109 as if fully set forth herein.

113. Defendants Richard Fuisz, Joseph Fuisz, and Fuisz Technologies knew that John Fuisz's Law Firm provided legal services to Plaintiffs and that John Fuisz was a member of the firm, and therefore knew that there were contractual obligations requiring the law firm, and John Fuisz as a partner, to maintain the confidentiality of Plaintiffs' Confidential Information and not to act contrary to Plaintiffs' interest.

114. With that knowledge, Defendants Richard Fuisz, Joseph Fuisz, and Fuisz Technologies intentionally induced John Fuisz to breach his contract with Plaintiffs.

115. Defendant John Fuisz did breach the contract when he misappropriated Plaintiffs' Confidential Information and furnished such Confidential Information to Defendants Richard Fuisz, Joseph Fuisz, and Fuisz Technologies.

116. The intentional inducement by Defendants Richard Fuisz, Joseph Fuisz, and Fuisz Technologies was a substantial factor in causing John Fuisz to breach his contract with Plaintiffs.

117. As a direct and proximate cause of Defendant John Fuisz's breach of contract, Plaintiffs have been harmed in an amount to be determined at trial and will continue to be harmed unless appropriate injunctive relief is granted.

Eleventh Claim
Civil Conspiracy
(Against All Defendants)

118. Plaintiffs repeat and reallege the allegations set forth in paragraphs 1 through 115 as if fully set forth herein.

119. Defendants comprise a group of persons who agreed to a common plan or design to commit a tortious act. Specifically, they agreed that John Fuisz would use his position as a member of John Fuisz's Law Firm to misappropriate Plaintiffs' Confidential Information and to furnish such Confidential Information to Defendants Richard Fuisz and Joseph Fuisz.

120. Pursuant to the agreement, John Fuisz used his position as a member of John Fuisz's Law Firm to misappropriate Plaintiffs' Confidential Information that enabled Defendants Richard Fuisz and Joseph Fuisz to obtain the '612 Patent.

121. Such an action harmed Plaintiffs in an amount to be determined at trial and will continue to harm Plaintiffs until appropriate injunctive relief is granted.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs seek judgment against Defendants as follows:

a. For an order to the PTO to correct the '612 Patent to add Elizabeth Holmes and Timothy Kemp as inventors;

b. For an order to the PTO to correct the '612 Patent to remove Richard Fuisz and Joseph Fuisz as inventors;

c. For general damages in an amount according to proof at trial;

d. For special damages in an amount according to proof at trial;

e. For punitive damages;

f. For appropriate injunctive relief;

g. For costs;

h. For reasonable attorney's fees;

i. For pre-judgment and post-judgment interest; and

j. For such other relief as the Court may deem appropriate.

JURY TRIAL DEMAND

Plaintiff hereby demands a trial by jury on all issues triable to a jury.

Dated: October 26, 2011

Respectfully submitted,

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EXHIBIT A

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
60/678,801	05/09/2005	Elizabeth Holmes	30696-702.101

CONFIRMATION NO. 8684

OC000000019007054

021971
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

Date Mailed: 05/30/2006

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/12/2006.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

MELKAM BEYENE
PTOSS (703) 305-3006

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APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
60/678,801	05/09/2005	Elizabeth Holmes	035738-0015

CONFIRMATION NO. 8684

OC000000019007013

20277
MCDERMOTT WILL & EMERY LLP
600 13TH STREET, N.W.
WASHINGTON, DC 20005-3096

Date Mailed: 05/30/2006

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/12/2006.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

MELKAM BEYENE
PTOSS (703) 305-3006

OFFICE COPY

Whereas, the undersigned:

1. Holmes, Elizabeth
Palo Alto, CA 94301

2. Roy, Shaunak
San Mateo, CA 94403

3. Howard, John
Saratoga, CA 95070

4. Wang, Chengwang
Mountain View, CA 94043

5. Gibbons, Ian
Portola Valley, CA 94028

6. Kemp, Tim
San Jose, CA 95120

hereinafter termed "Inventors", have invented certain new and useful improvements in

SYSTEM FOR REAL-TIME THERAPEUTIC MONITORING

☒ for which an application for United States Patent was filed on May 9, 2005, Application No. 60/678,801.
☐ for which a United States Patent issued on ___, U.S. Patent No. ___.

WHEREAS, Theranos, Inc., a corporation of the State of Delaware, having a place of business at 1430 O'Brien Drive, Suite H, Menlo Park, CA 94025, (hereinafter termed "Assignee"), is desirous of acquiring the entire right, title and interest in and to said application and the invention disclosed therein, and in and to all embodiments of the invention, heretofore conceived, made or discovered jointly or severally by said Inventors (all collectively hereinafter termed "said invention"), and in and to any and all patents, inventor's certificates and other forms of protection (hereinafter termed "patents") thereon granted in the United States and foreign countries.

NOW, THEREFORE, in consideration of good and valuable consideration acknowledged by said Inventors to have been received in full from said Assignee:

1. Said Inventors do hereby sell, assign, transfer and convey unto said Assignee the entire right, title and interest (a) in and to said application and said invention; (b) in and to all rights to apply for foreign patents on said invention pursuant to the International Convention for the Protection of Industrial Property or otherwise; (c) in and to any and all applications filed and any and all patents granted on said invention in the United States or any foreign country, including each and every application filed and each and every patent granted on any application which is a divisional, substitution, continuation, or continuation-in-part of any of said applications; and (d) in and to each and every reissue or extensions of any of said patents.

2. Said Inventors hereby jointly and severally covenant and agree to cooperate with said Assignee to enable said Assignee to enjoy to the fullest extent the right, title and interest herein conveyed in the United States and foreign countries. Such cooperation by said Inventors shall include prompt production of pertinent facts and documents, giving of testimony, execution of petitions, oaths, specifications, declarations or other papers, and other assistance all to the extent deemed necessary or desirable by said Assignee (a) for perfecting in said Assignee the right, title and interest herein conveyed; (b) for prosecuting any of said applications; (c) for filing and prosecuting substitute, divisional, continuing or additional applications covering said invention; (d) for filing and prosecuting applications for reissuance of any said patents; (e) for interference or other priority proceedings involving said invention; and (f) for legal proceedings involving said invention and any applications therefor and any patents granted thereon, including without limitation reissues and reexaminations, opposition proceedings, cancellation proceedings, priority contests, public use proceedings, infringement actions and court actions; provided, however, that the expense incurred by said Inventors in providing such cooperation shall be paid for by said Assignee.

3. The terms and covenants of this assignment shall inure to the benefit of said Assignee, its successors, assigns and other legal representatives, and shall be binding upon said Inventors, their respective heirs, legal representatives and assigns.

4. Said Inventors hereby jointly and severally warrant and represent that they have not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

IN WITNESS WHEREOF, said Inventors have executed and delivered this instrument to said Assignee as of the dates written below:

Date: April 19, 2006 — Elizabeth Holmes

Date: 04/28/06 — Shaunak Roy

Date: 5/31/06 — John Howard

Date: 4/25/2006 — Chengwang Wang

Date: 04/25/06 — Ian Gibbons

Date: 04/28/2006 — Tim Kemp

Practitioner's Docket No.: 30696-702.101 ***PATENT***

POWER OF ATTORNEY BY ASSIGNEE TO EXCLUSION OF INVENTOR UNDER 37 C.F.R. § 3.71 WITH REVOCATION OF PRIOR POWERS

The undersigned ASSIGNEE of the entire interest in:

☐ U.S. Patent No.
☒ U.S. application no. 60/678,801, filed on May 9, 2005

hereby appoints all Wilson Sonsini Goodrich & Rosati attorneys registered to practice before the United States Patent and Trademark Office, as associated with:

Customer No. 021971

to prosecute this application and transact all business in the United States Patent and Trademark Office in connection therewith and hereby revokes all prior powers of attorney; said appointment to be to the exclusion of the inventors and the inventors' attorneys in accordance with the provisions of 37 C.F.R. § 3.71.

The following evidentiary documents establish a chain of title from the original owner to the Assignee:

(complete one of the following)

☒ a copy of an Assignment attached hereto, which Assignment has been (or is herewith) forwarded to the Patent and Trademark Office for recording; or

☐ the Assignment recorded on ___ at reel ___, frames ___-___.

Pursuant to 37 C.F.R. § 3.73(b) the undersigned Assignee hereby states that evidentiary documents have been reviewed and hereby certifies that, to the best of ASSIGNEE's knowledge and belief, title is in the identified ASSIGNEE.

CHANGE OF CORRESPONDENCE ADDRESS

Direct all correspondence and telephone calls to:

Name	Karen K. Wong, Ph.D. J.D.					
Address	Wilson Sonsini Goodrich and Rosati					
Address	650 Page Mill Road					
City	Palo Alto	State	CA	Zip	94304	Customer No.: 021971
Country	USA	Telephone	(650) 493-9300	Fax	(650) 493-6811	

ASSIGNEE: Theranos, Inc.

Name: Elizabeth Holmes
Print

Signature

Title: President and CEO

Date: April 19, 2006

Electronic Acknowledgement Receipt

EFS ID:	1045959
Application Number:	60678801
Confirmation Number:	8684
Title of Invention:	System for real-time therapeutic monitoring
First Named Inventor:	Elizabeth Holmes
Customer Number:	20277
Filer:	Vernon A. Norviel/cathy bachmann/VN/KW/CB
Filer Authorized By:	Vernon A. Norviel
Attorney Docket Number:	035738-0015
Receipt Date:	12-MAY-2006
Filing Date:	09-MAY-2005
Time Stamp:	14:21:23
Application Type:	Provisional
International Application Number:	

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)	Multi Part	Pages
1	Power of Attorney (may include Associate POA)	30696-702-101POA.pdf	122969	no	2

Warnings:	
Information:	
Total Files Size (in bytes):	122969

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Express Mail Label No.			Docket Number	035738-0015

INVENTOR(s)/APPLICANT

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (City and Either State or Foreign Country)
HOLMES	**Elizabeth**		**San Francisco, CA**
ROY	**Shaunak**		**San Mateo, CA**
HOWARD	**John**		**Saratoga, CA**
WANG	**Chengwang**		**Mountain view, CA**
GIBBONS	**Ian**		**Portola Valley, CA**
KEMP	**Tim**		**San Jose, CA**

Additional inventors are being named on the separately numbered sheets attached hereto.

TITLE OF THE INVENTION (500 characters max)

SYSTEM FOR REAL-TIME THERAPEUTIC MONITORING

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ENCLOSED APPLICATION PARTS ***(check all that apply)***

☒ Specification *Number of pages* [15] ☒ Small Entity Statement
☒ Drawings *Number of sheets* [17] ☐ Other (specify): _____

Application Size Fee: If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(I)(G) and 37 CAR 1.16(s).

METHOD OF PAYMENT OF APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.

TOTAL FEE ($)

☐ A check or money order is enclosed to cover the filing fee and application size fee (if applicable). **$100.00**

☒ The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number: 500417 .

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

McDERMOTT WILL & EMERY LLP

Thomas A. Haag, Ph.D., Esq.
Registration No. 47,621

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SYSTEM FOR REAL-TIME THERAPEUTIC MONITORING

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background of the invention

1. Field of the Invention

[0001] This invention relates generally to real-time therapeutic monitoring. More particularly, it relates to customized clinical decision support systems.

[0002] 2. Description of the Related Art

[0003] The safety and efficacy of a drug is determined by the pharmacokinetic (what the body does to the drug) and pharmacodynamic parameters (what the drug does to the body) of the drug. Currently, the pharmacokinetic (PK) and pharmacodymanic (PD) parameters of a drug are generally determined by drawing blood samples from a patient at pre-determined time periods and analyzed in a laboratory setting. As can be easily understood, such an approach has numerous shortcomings. The patient has to be generally confined to a clinical setting so that blood samples could be drawn at periodic intervals; hence, ambulatory, real-time continuous monitoring becomes almost impossible.

[0004] Currently, most of the analytical techniques for determining target analyte and biomarker concentrations require that whole blood be pre-processed for analysis. This results in problems of time lags in data response, variability in physiological drug distribution and metabolism (warranting poor dosing), sparse sampling, and the lack of dosing history. Additionally, current clinical trial protocols often suffer from insufficient numbers of blood tests from patients, because they must return to a phlebotomist for blood samples to be drawn throughout the trial.

[0005] As a consequence of the inability to do real-time continuous monitoring in ambulatory patients, PK and PD effects that happen on a shorter time scale become almost impossible to determine. More importantly, adverse drug reactions (ADRs) are not identified until it is too late. Determining ADRs in individual patients or drug-drug interactions at an early

stage of a clinical trial is extremely valuable for patient safety, as well as effective drug development. Continuous analyte and biomarker profiling of a patient's blood is critical to managing the risk of ADRs. Current technologies are unable to provide this capability of quantifying and monitoring selected analytes in real time.

[0006] Furthermore, currently there is no cost-effective way of comparing in real-time the PK and PD parameters of a drug, with respect to an individual patient, against a normalized PK and PD profile of an ideal patient. Such comparisons would assist in the early identification of the potential for ADRs and drug-drug interactions. Additionally, such parameters are not available from ambulatory patients; certainly not using any economically feasible means. Also, there are no means currently available for determining such parameters from a very small sample of blood, e.g., less than 50μl.

[0007] Thus, there is a need for a system and methods for determining the PK and PD parameters of a drug in ambulatory patients using small volumes of blood, particularly in real-time and *in situ* (as opposed to a analyzing the sample in a clinical laboratory). Additionally, it is desirable to profile targeted analytes and biomarkers in small blood samples of patients. Such profiling could assist in determining ADRs and undesirable drug-drug interactions.

SUMMARY OF THE INVENTION

[0008] The present invention overcomes the limitations of the prior art by providing a disposable system that is capable of drawing microliter quantities of blood in ambulatory patients and determining *in situ* and/or real-time the concentrations of targeted analytes and biomarkers. Additionally the system has the capability of engaging in two-way communication with external databases and other data repositories.

[0009] A system for monitoring certain targets in blood comprising a disposable unit that is, preferably wirelessly, integrated with a two-way communication device, wherein the disposable unit has (a) means for sampling blood in volumes less than 50μl, (b) means for determining the concentration of targeted analytes and biomarkers in the blood, and (c) is capable of transferring data that are related to the concentration of the targeted analytes in the blood signals to the communication device.

[0010] A method of monitoring the pharmacokinetic and pharmacodynamic parameters of a drug in a patient by (a) measuring the concentration of one or more target drugs and their analytes in microliter quantities of the patient's blood, and (b) measuring pharmacodynamic parameters related to one or more of the target drugs and their metabolites, wherein the pharmacodynamic parameters include vitals of the patient and one or more biomarkers that are influenced by the presence of the one or more drugs and their metabolites.

[0011] A method of continuously and simultaneously measuring, in humans, multiple analytes including drugs, metabolites and biomarkers in whole blood using a system that is capable of drawing less than 50μl of blood and detecting *in situ* one or more analytes.

[0012] A handheld medical device for measuring pharmacokinetic and pharmacodynamic parameters of a drug comprising a blood sample acquisition cartridge employing microfluidics for separating and moving the sample around, an assay layer that is functionally linked to the

cartridge and capable of analyzing specific analytes and biomarkers in the blood sample, a reader that is functionally linked to the assay layer and capable of reading signals emanating from the assay layer, a communication chip that is functionally linked to the reader and communicates the output from the reader to a communication network.

[0013] In an alternate embodiment, a system capable of simultaneously measuring multiple analytes in a small sample of whole blood, wherein the blood sample includes one or more drugs, metabolites of the drug(s) and biomarkers, where the blood sample is less than 50μl.

[0014] Other aspects of the invention include methods corresponding to the devices and systems described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The invention has other advantages and features which will be more readily apparent from the following detailed description of the invention and the appended claims, when taken in conjunction with the accompanying drawings, in which:

[0016] FIG. 1A is a schematic of the Metabolic Profiler that provides pharmacokinetic and pharmacodynamic parameters related to one or more drugs of interest; FIG. 1B is a schematic of the capabilities of a managed network for real-time therapeutic monitoring.

[0017] FIG. 2A shows the capabilities of the Metabolic Profiler; FIG. 2B shows the PK and PD data that are provided by the Metabolic Profiler

[0018] FIG. 3. is a schematic of how the Metabolic Profiler interacts with external data.

[0019] FIG. 4A is a flowchart showing the data flow and the actions taken based on patient's PK and PD data; FIG. 4B shows how the centralized clinical data could be shared with entities who are involved in the care of the patient.

[0020] FIG. 5 shows the different technologies that could be embedded in the Metabolic Profiler.

[0021] FIG. 6 illustrates the difference between competitive binding and two step immunoassay formats.

[0022] FIG. 7 is an illustration of the chemiluminescence enzyme immunoassay incorporating the two-step method.

[0023] FIG. 8 is a dose response of two-step chemuiluminescence assay compared to competitive binding immunoassays.

[0024] FIG. 9 illustrates the effect of plasma on competitive binding and two step immunoassay.

[0025] FIG. 10 illustrates the enhancement in sensitivity in a two step assay process.

[0026] FIG. 11 shows the capability to simultaneously measure low and high concentrations in the same sample.

[0027] FIG. 12 shows an illustration of a cartridge containing a fluid layer, reagents, access and bleed ports.

[0028] FIG. 13 is an illustration of the fluid flow in the cartridge.

[0029] FIG. 14 is a block diagram of the PC Card in the device.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0030] Although the detailed description contains many specifics, these should not be construed as limiting the scope of the invention but merely as illustrating different examples and aspects of the invention. It should be appreciated that the scope of the invention includes other embodiments not discussed in detail above. Various other modifications, changes and variations which will be apparent to those skilled in the art may be made in the arrangement, operation and details of the method and apparatus of the present invention disclosed herein without departing from the spirit and scope of the invention as described here.

[0031] FIG. 1 illustrates a handheld metabolic profiler. Generally, the profiler comprises a cartridge, an assay assembly, a reader assembly and an input-output (I/O) communication assembly. A lancet is launched from the handheld to draw a small volume of blood from a suitable anatomical site, such as the tip of a finger of the ventral forearm. The volumes are typically in the less than 100 µl range and preferably less than 50µl. In the cartridge assembly, the blood sample that is drawn would then undergo many processing steps that employ microfluidics. Once the components of the blood sample are isolated, a drug specific analyte (concentration of the drug and/or metabolite(s) of the drug) is measured using various assays that are built in to the hand held. The handheld also has capabilities of wireless communicating with the external world. The handheld could transmit the results of the analysis to an external database and is also capable of receiving data from such databases. Some of the features regarding sampling and analytical methods have been disclosed in an earlier filed application by the same inventor now published as WO 2005/025413A2, which is incorporated by reference in its entirety for all purposes.

[0032] The metabolic profiler could also be worn on the body instead of being held in hand. The device could have an adhesive backing or other means of remaining on the body surface of a patient.

[0033] FIG. 2A shows a sample list of the parameters that the metabolic profiler is capable of measuring and communicating: for example, concentration in the sampled blood of one or more drugs that are of interest, the metabolite(s) of those drug(s); biomarkers such as proteins and cells in the blood; vitals such as temperature, heart and respiratory rate and blood pressure. The biomarkers could be indicative of disease or could be a result of the action of the drug(s). Fig. 2B illustrates the commonly understood pharmacokinetic and pharmacodynamic parameters related to a drug. The pharmacokinetic (PK) parameters, the concentration of a drug and its metabolites in blood as a function of time is a critical parameter in appropriate dosing of a patient. Identifying and quantifying the PK parameters in real time, from a sample volume is extremely desirable for proper safety and efficacy of drugs. If the drug and metabolite concentrations result in outside the desired range and/or unexpected metabolites are generated, due to an unexpected reaction to the drug, immediate action might have to be taken to ensure the safety of the patient. Similarly, if any of the pharmacodynamic (PD) parameters that are shown in Fig. 2B (vitals or biomarkers) fall outside the desired range during a treatment regime, immediate action may have to be taken as well.

[0034] FIG. 3 shows a schematic of how the metabolic profiler assists in ensuring the safety and efficacy of a drug. The RDx metabolic profiler is capable of collecting PK and PD data of one or more drugs related to a particular patient. It is wirelessly linked, either through a transmitter or other commonly known means, to a computer network. The computer network hosts a variety of databases, which contain information relating the individual patient, pharmacogenomic data and the like. The data transmitted by the metabolic profiler is analyzed using appropriate algorithms and a conclusion about the measured PK and PD parameters is reached. This conclusion is then transmitted to the appropriate recipients. The process is described in further detail in Fig. 4

[0035] FIG. 4 shows a flowchart starting with the patient. Either using a handheld profiler or one that is worn, the profiler gathers PK and PD parameters related to a patient of interest. As

described above, these data are then securely transmitted over, for example, a cellular network or the internet, and interpretations of the data are derived through computations in a series of biostatistical algorithms on the server which correlate pharamcodynamic, pharmacokinetic, and pharmacogenetic profiles. Additionally, the data can be compared with information stored in databases. The stored information could be the patient's own PK and PD data over the previous treatment regiment, it could be data related to placebo, or it could be pharmacogenomic data that are of relevance to the particular patient. If the analysis done in Step 2 suggests that there are no significant difference between the patient's data and the stored data, as determined by using appropriate algorithms, then we arrive at Step 3—"No Action" is taken. However, the outcome of the analysis in Step 2 is that there is a significant difference, then Step 4 determines how large the difference is. If it is a large difference, immediate action is taken. One kind of immediate action could be to provide an emergency alert to the patient's healthcare provider. Another kind of immediate action would be to send instructions to the profiler to alter the dosing of the drug, if the profiler has the built-in drug delivery capability. If the conclusion of the Step 4 analysis is the difference is small, then the algorithm would determine whether Steps 6 or 7 should be executed: continue monitoring the parameters and/or alter dose.

[0036] One of the significant advantages of the envisioned network is illustrated in Fig. 4B. As all the information is securely channeled through the internet, this allows the simultaneous sharing of the information with various interested parties, while satisfying the appropriate clinical, regulatory and business needs. For example, the flowchart in Fig. 4A shows how the patient's clinical needs are met. The ability of the company that is sponsoring a drug study, for example a clinical trial or a post-market Phase IV surveillance, to monitor in real-time the safety and efficacy of the performance of the drug provides extremely valuable regulatory and business information. Similarly, the ability of a payor to monitor the efficacy (and perhaps cost-effectiveness) of a treatment is greatly enhanced by their ability to obtain data in real-time.

[0037] FIG. 5 lists the various technologies that are incorporated in the metabolic profiler. The small volume blood sampling and processing those small volumes is effectively accomplished using microfluidics. The other component of the profiler is assaying the analytes—drugs, metabolites and biomarkers. Various commercially available ligands or specially developed for specific analytes could be used. Measurement techniques based on fluorescence, chemiluminescence, chemiluminescence amplified by enzyme linked immunoassay, etc. can be used to identify and quantify the analytes.

[0038] **Two-Step Protocol to Perform Immunoassays in a Handheld Device**

[0039] In a laboratory, small molecules are most commonly assayed via a competitive binding method. This requires the mixing of the conjugate with the sample before exposing the mixture to a surface bearing the antibody. In a small medical device, this would be a very difficult as it requires that the reagents be well mixed with the sample matrix prior to exposure to the antibodies. A two-step method eliminates the need for mixing as well as any interference between the conjugate and the sample.

[0040] For ease of integration into a portable device, a two-step assay has significant advantages over the more common laboratory standard immunoassays (competitive binding). It combines the ease of use and high sensitivity of a sandwich immunoassay with the ability to assay small molecules.

[0041] The two-step assay is run as shown in FIG. 6. First the sample containing analyte (marked Ag) is flowed over a surface containing antibodies (denoted by "Y"). The antibodies bind the analyte out of the sample. Based on the antibody affinity, the surface will adsorb various amounts of the analyte, as in a standard sandwich immunoassay. After the sample passes over the surface, a solution with analyte conjugated to a marker (labeled Ag) at a high concentration is passed over. The conjugate saturates any of the antibodies that have not yet bound the analyte. Before equilibrium is reached and any displacement of pre-bound unlabelled

analyte occurs, the high-concentration conjugate solution is washed off. The amount of conjugate bound to the surface is then measured by the appropriate technique.

[0042] One such appropriate measure technique is chemiluminescence enzyme immunoassay. This is a modified form of a commercially available chemiluminescence enzyme immunoassay. The enzyme used is alkaline phosphatase and the substrate is a commercially available dioxitane-phosphate, which is not luminescent but becomes luminescent after hydrolysis by alkaline phosphatase. The substrate solution is supplemented with "enhancing agents," which create a much brighter signal than the luminophore alone. Moreover, an alkaline phosphatase conjugate with a higher turnover number than that used in the commercial assay is employed. This allows signal generation to proceed much more rapidly and a higher overall signal is achieved. This assay is illustrated in FIG. 7. The increased sensitivity of the two-step chemiluminescent enzyme immunoassay (TOSCA) is illustrated in FIG. 8. FIG. 8 shows that for analytes in the picomolar concentration, TOSCA is able to provide a more robust signal (higher sensitivity) than the competitive binding assay.

[0043] Additionally, TOSCA is less sensitive to matrix effects than other methodologies. This allows one to work with samples that have not been extensively pre-processed using standard laboratory techniques such as dilution, solid phase extraction, chromatography, etc. The ability of TOSCA to assay less than ideal samples and maintain desired sensitivity is illustrated in FIG. 9. Compared to competitive binding assay, for all sample preparations (and dilutions), TOSCA has better sensitivity than competitive binding. This is also illustrated in FIG. 10 where the sensitivity enhancement achieved using TOSCA is compared with the two-step assay.

[0044] **Simultaneous High and Low Sensitivities using TOSCA**

[0045] Currently, there are no effective techniques that one could use in a hand held analyzer where the analytes are present in widely varying concentration range, e.g., one analyte

is in the pg/ml concentration and the other is in the ng/ml concentration. TOSCA has the ability to simultaneously assay analytes that are present in the same sample in a wide concentration range. FIG. 11 shows two analytes, prostacyclin metabolite and thromboxane metabolite, that have been identified and quantified when their concentrations are different by more than 3 orders of magnitude. Another useful application for the ability to measure concentration of different analytes that are present in a wide concentration range is relating the ratios of the concentration of these analytes to safety and efficacy of multiple drugs administered to a patient. Frequently, unexpected drug-drug interactions are a common cause of adverse drug reactions. A real-time, concurrent measurement technique for measuring different analytes would help avoid the potentially disastrous consequence of drug-drug interactions. Furthermore, knowing the change in ratios of different analytes and computing the rate of those changes, whether they are drugs or their metabolites, would help ward off potentially dangerous situations before they happen. For example, if glucose were the analyte (or marker) one were measuring, not only the concentration of glucose at any one instant, but the rate of change of the glucose concentration is highly useful in predicting and avoiding, for example, hypoglycemic events. Such a trend analysis has widespread beneficial implications in drug dosing regimen. Particularly when multiple drugs and their metabolites are concerned, the ability to spot a trend and take proactive measures is very desirable.

[0046] Cartridge for Hand-held (or Worn) Profiler

[0047] FIG. 12 shows a schematic for a fully self-contained cartridge that enables the metabolic profiler to receive microliter volume of sampled blood, separate the components of the blood using microfluidics and mix the separated streams with reagents on board. The cartridge has access ports for infusing materials, collecting waste and interacting with a reader. The fluid flow in such a cartridge is illustrated in FIG. 13. The cartridge interfaces with a reader that is capable of registering the results of the analyte assays. The cartridge and the reader are together controlled by a controller. The controller contains a communication chip and controls the

photosensors that measure the signals from the assay (such as TOSCA), the pump and valve drivers of the cartridge. A schematic of this is illustrated in FIG. 14.

SYSTEM FOR REAL-TIME THERAPEUTIC MONITORING

ABSTRACT OF THE DISCLOSURE

A miniature system that could be either worn by a patient or held in hand for measuring pharmacokinetic and pharmacodynamic parameters of one or more drugs is disclosed. The system is capable of measuring drugs, metabolites and biomarkers in small volumes of blood. These measurements are made in real time and can be communicated to computer networks housing databases, where the individual patient's data are analyzed using appropriate algorithms and information stored in such databases. This enables real-time monitoring of safety and efficacy of drugs in patients.

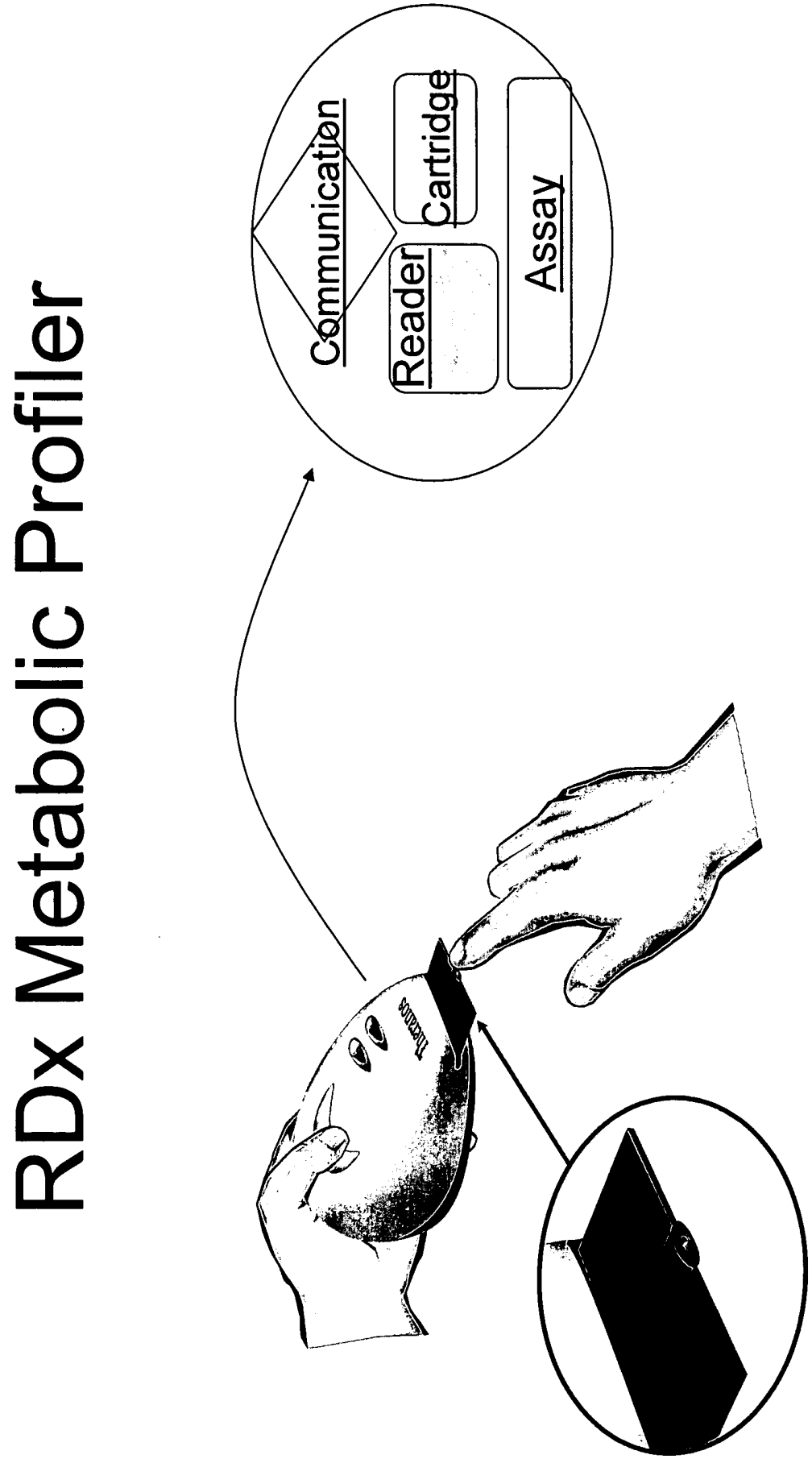

RDx Metabolic Profiler
Communication
Reader
Cartridge
Assay

FIG. 1A

Managed Network for Real-Time Therapeutic Monitoring

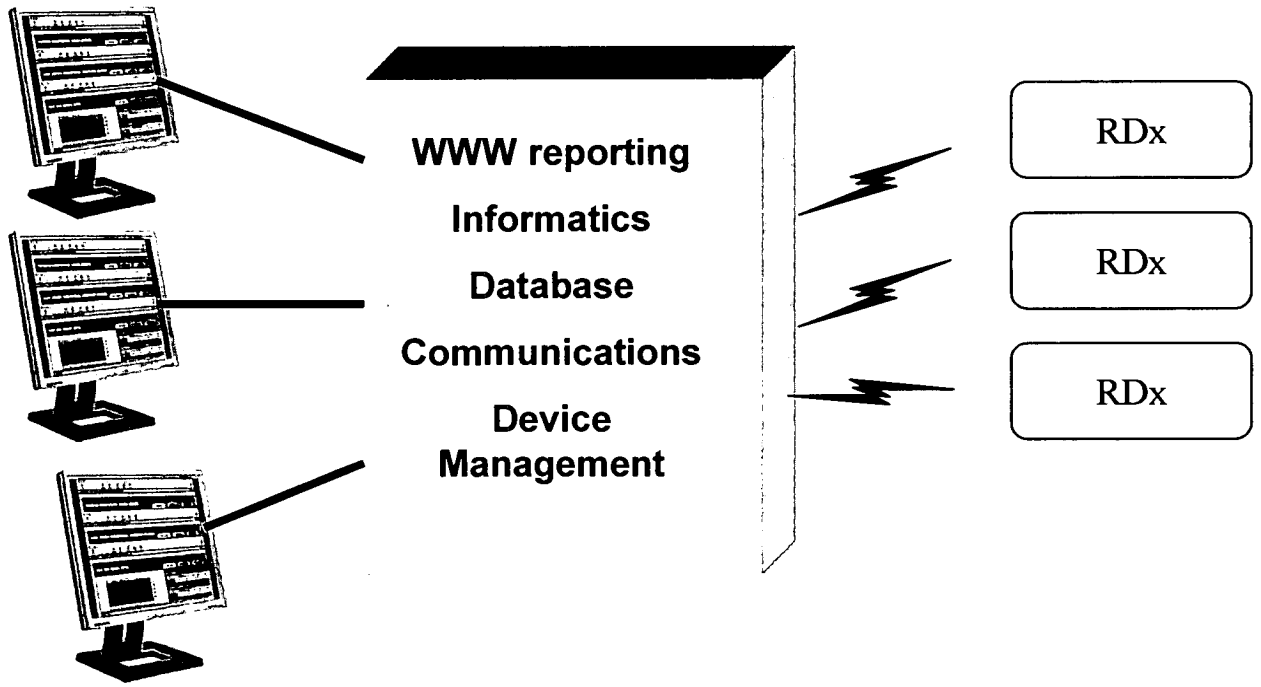


FIG. 1B

Theranos RDx Metabolic Profiler

Device capable of measuring, transmitting and receiving instructions

- Analyte Concentration
  - Drugs
  - Metabolites
- Biomarkers
  - Expressed Proteins
  - Cell markers
- Vitals
  - Temperature
  - Heart rate
  - Respiratory rate
  - Blood pressure

FIG. 2A

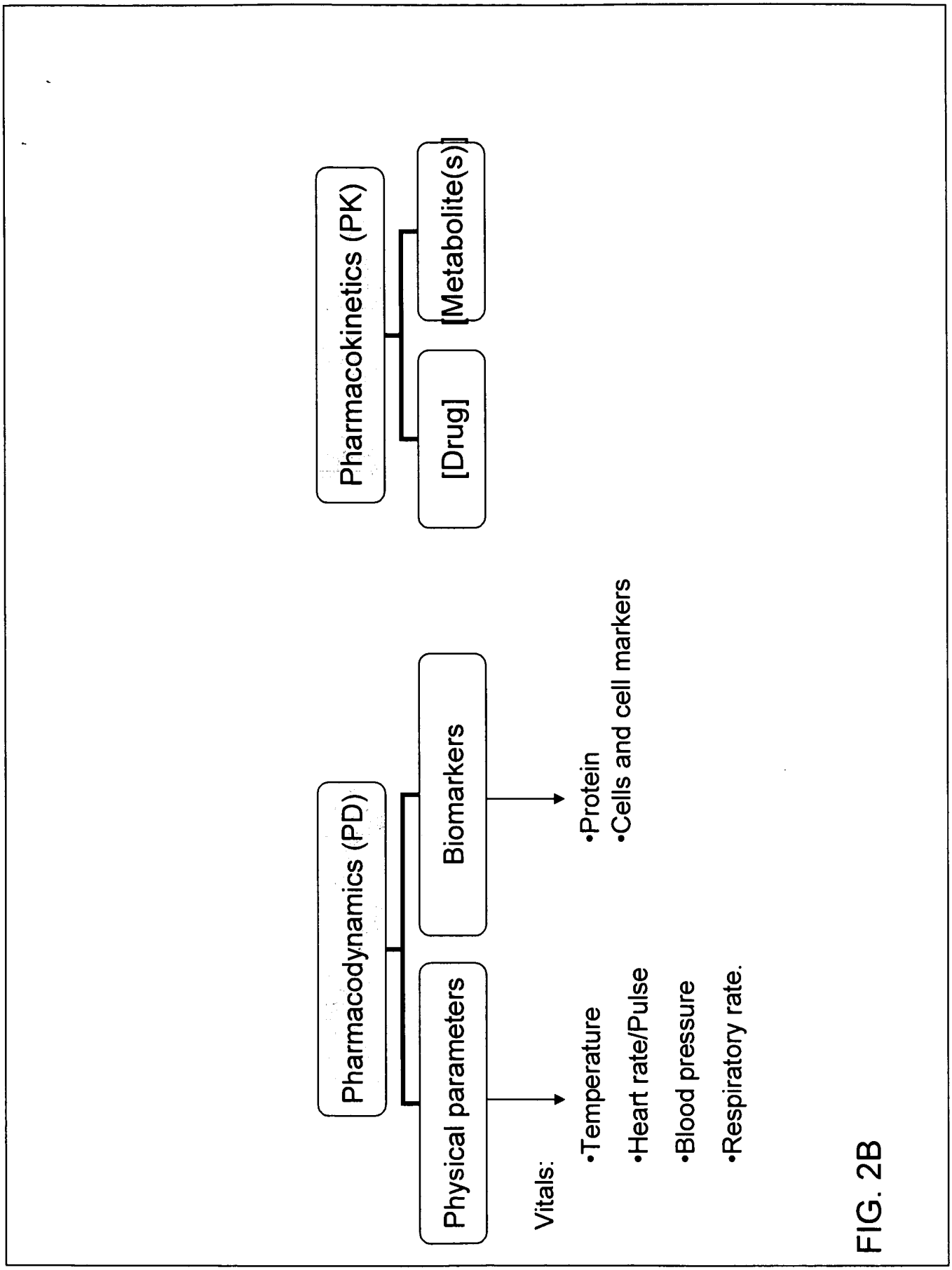

Pharmacokinetics (PK)
[Drug]
[Metabolite(s)]
Pharmacodynamics (PD)
Physical parameters
Biomarkers
Vitals:
•Temperature
•Heart rate/Pulse
•Blood pressure
•Respiratory rate.
•Protein
•Cells and cell markers

FIG. 2B

RDx Metabolic Profiler Operation

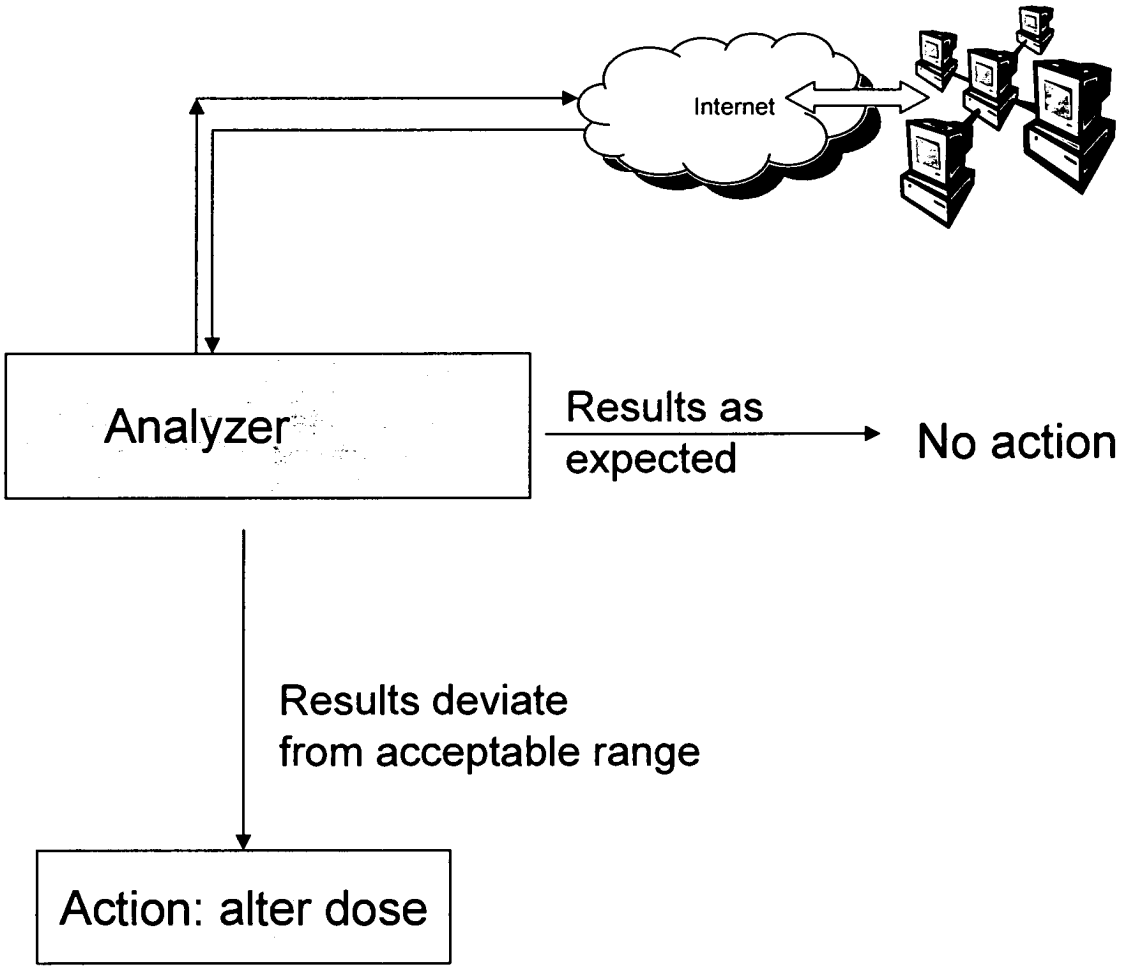
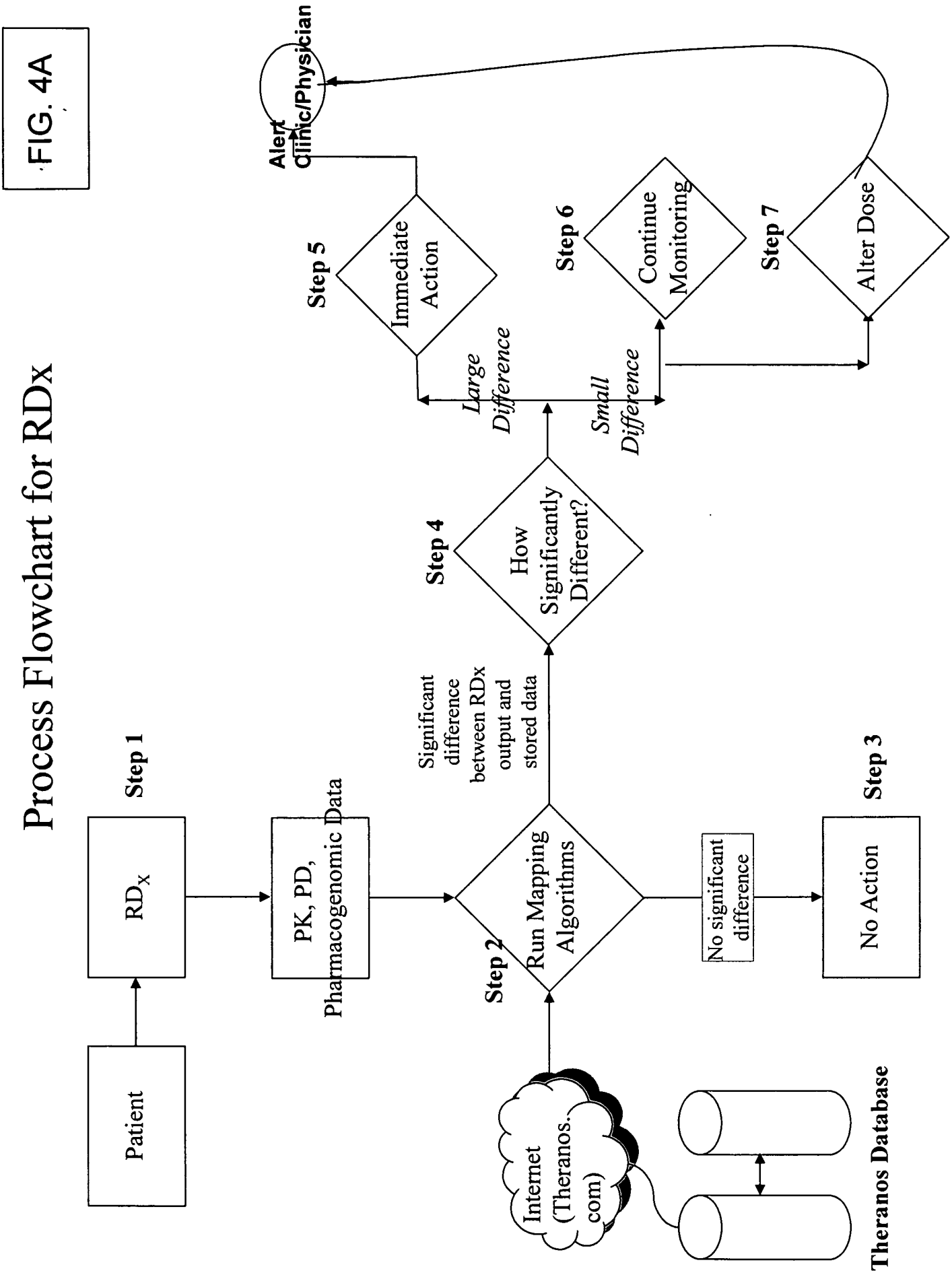


FIG. 3

FIG. 4A


Process Flowchart for RDx
Patient
RDx
Step 1
PK, PD, Pharmacogenomic Data
Run Mapping Algorithms
Step 2
No significant difference
No Action
Step 3
Significant difference between RDx output and stored data
How Significantly Different?
Step 4
Large Difference
Small Difference
Immediate Action
Step 5
Continue Monitoring
Step 6
Alter Dose
Step 7
Alert Clinic/Physician
Internet (Theranos.com)
Theranos Database

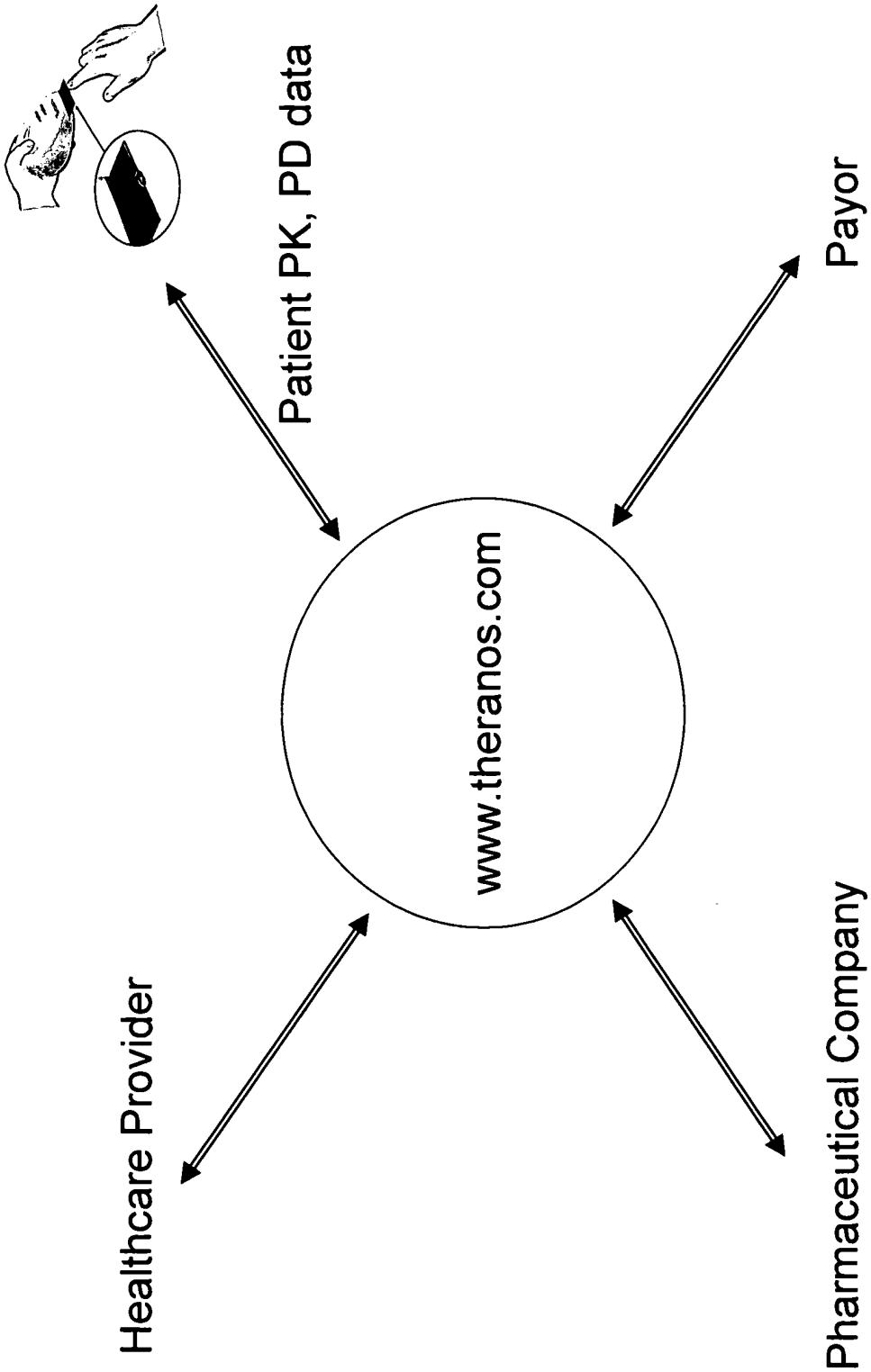

Patient PK, PD data
Payor
www.theranos.com
Healthcare Provider
Pharmaceutical Company

FIG. 4B

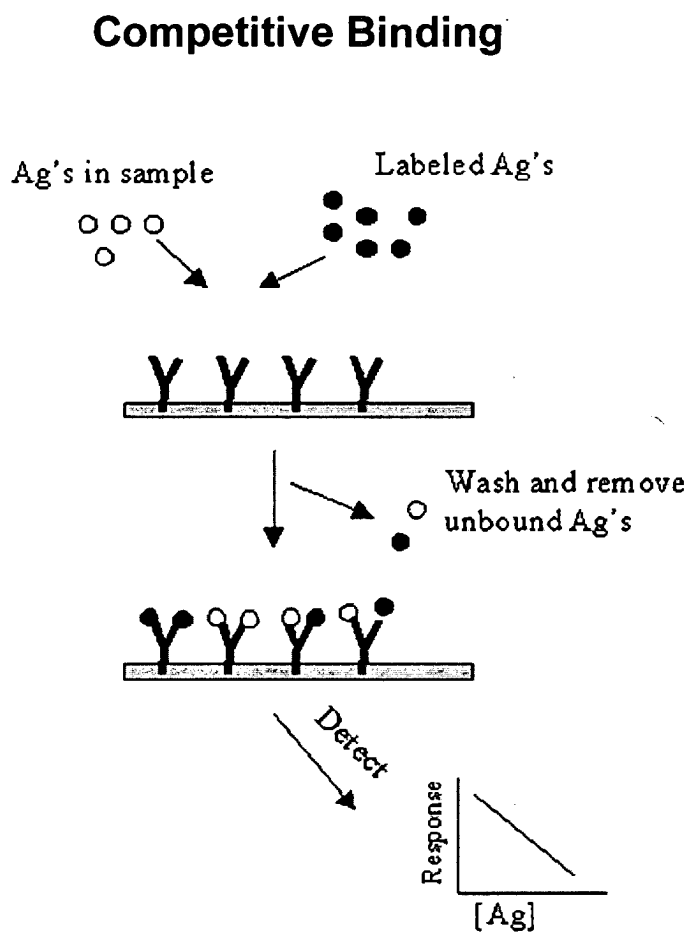
Technologies Incorporated in RDx

- Blood sampling
  - » Microfluidics
- Ligands that bind to analytes & biomarkers
- Analyte and biomarker measurement techniques
  - » Fluroscence
  - » Laminar flow assay
  - » Chemiluminescence
  - » Chemiluminescence amplified by EI
  - » Direct sensing (nanosensors)

PK
PD
»Pharmacogenomics (microPCR)

FIG. 5

Immunoassay Formats



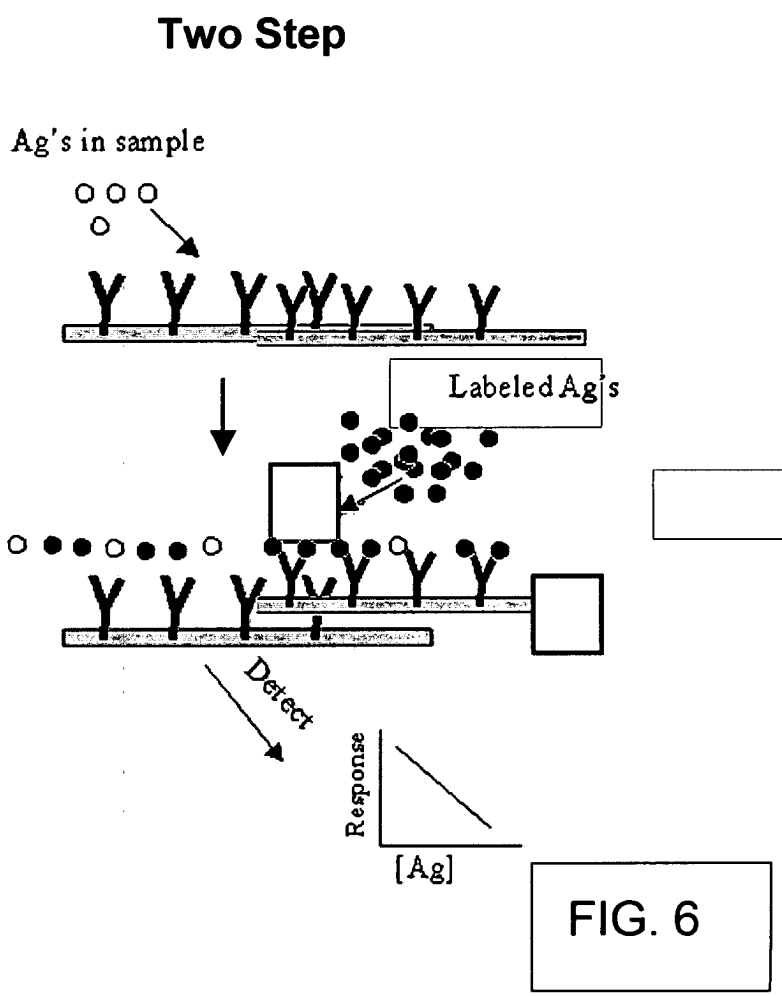


FIG. 6

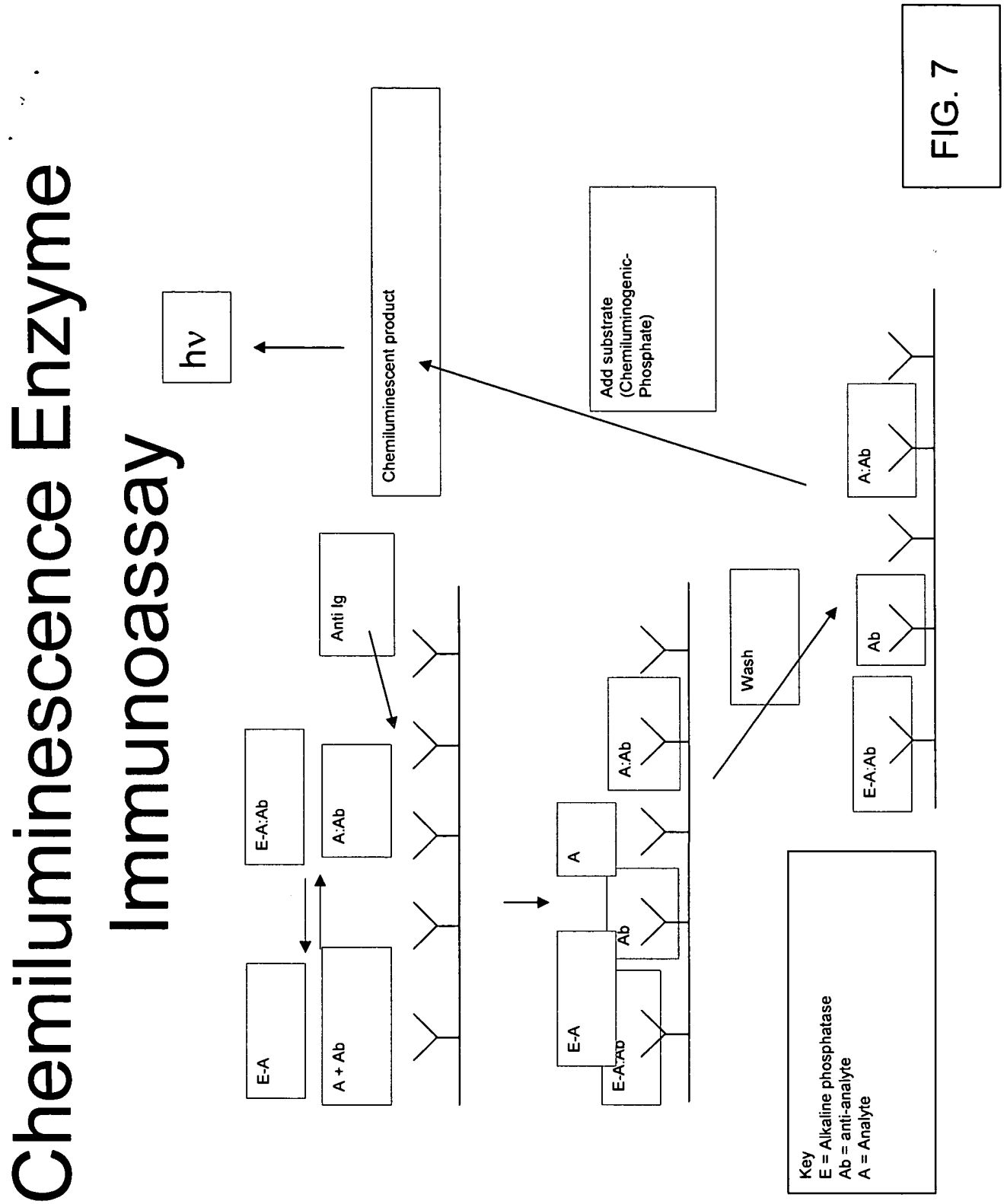
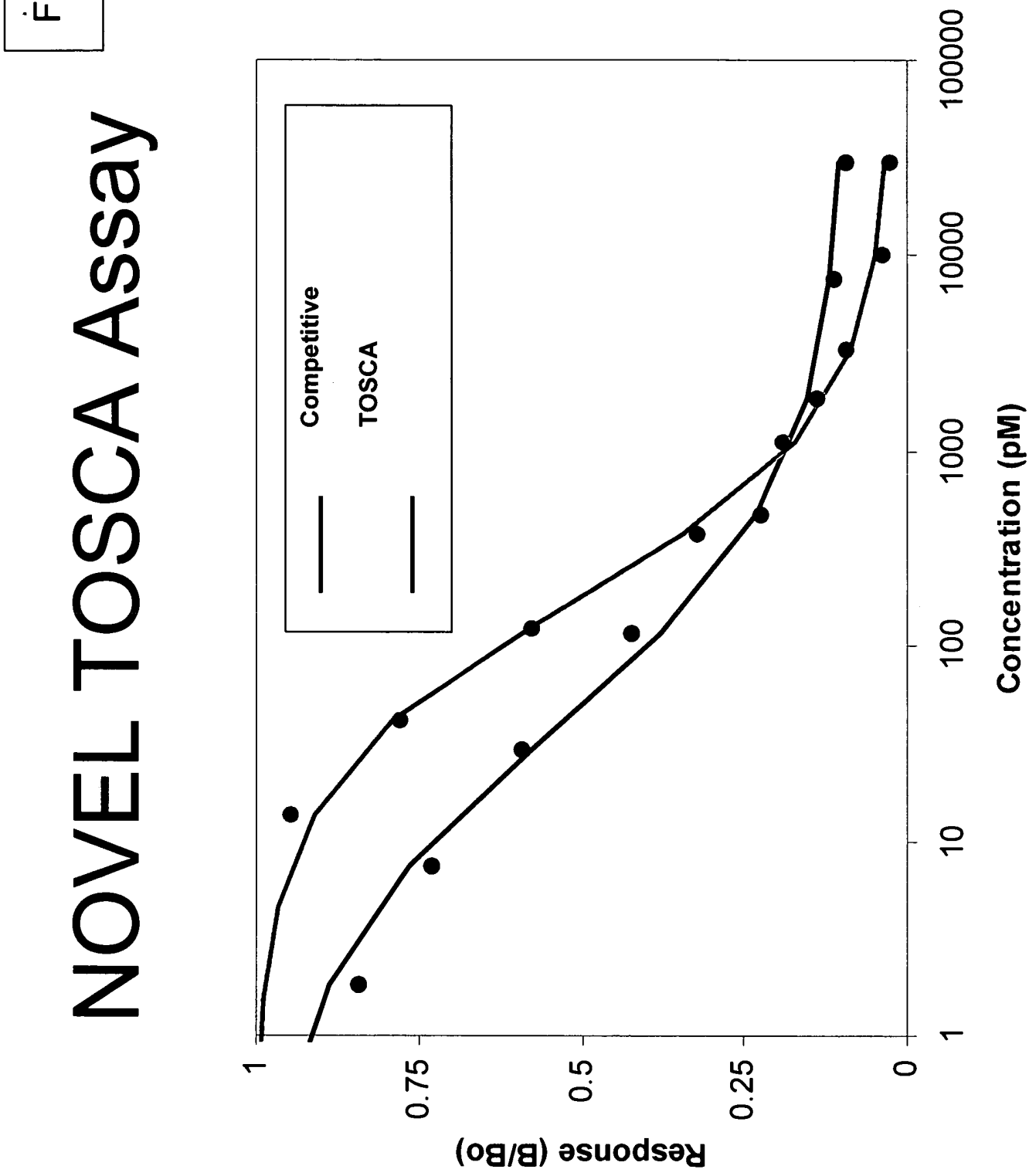
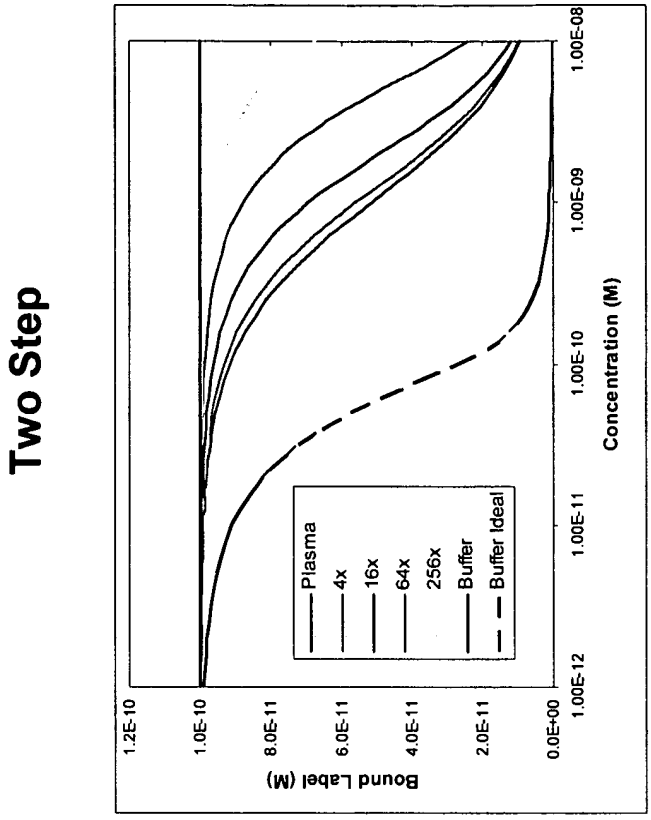

Chemiluminescence Enzyme Immunoassay
hν
Chemiluminescent product
Add substrate (Chemiluminogenic-Phosphate)
Anti Ig
E-A:Ab
A:Ab
E-A
A + Ab
A
A:Ab
E-A
Ab
E-A:Ab
Wash
A:Ab
Ab
E-A:Ab
Key
E = Alkaline phosphatase
Ab = anti-analyte
A = Analyte

FIG. 7

FIG. 8


NOVEL TOSCA Assay
Competitive
TOSCA
Response (B/Bo)
0
0.25
0.5
0.75
1
Concentration (pM)
1
10
100
1000
10000
100000

Plasma Effect



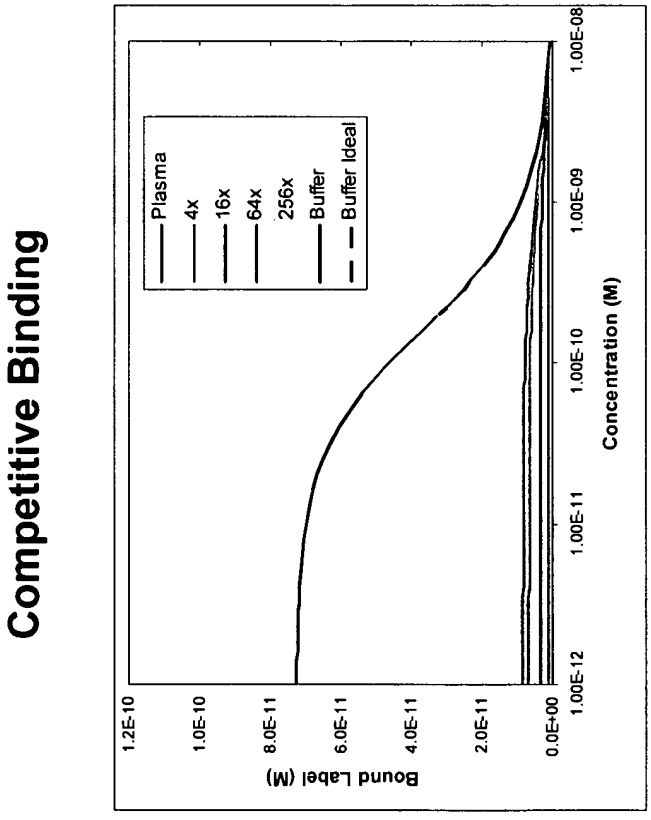


FIG. 9

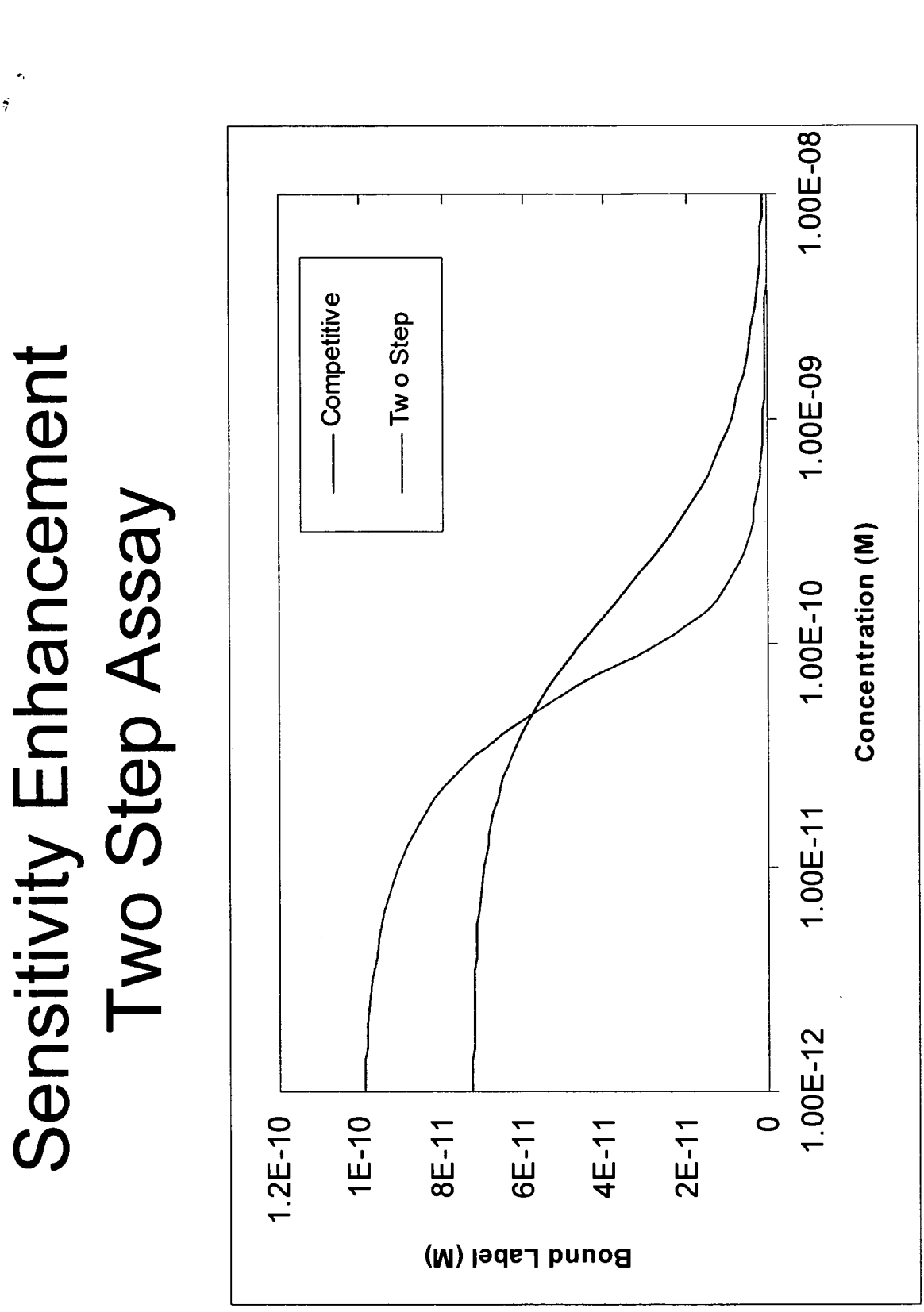

Sensitivity Enhancement
Two Step Assay
Competitive
Tw o Step
Bound Label (M)
1.2E-10
1E-10
8E-11
6E-11
4E-11
2E-11
0
1.00E-12
1.00E-11
1.00E-10
1.00E-09
1.00E-08
Concentration (M)

FIG. 10

Theranos Assay

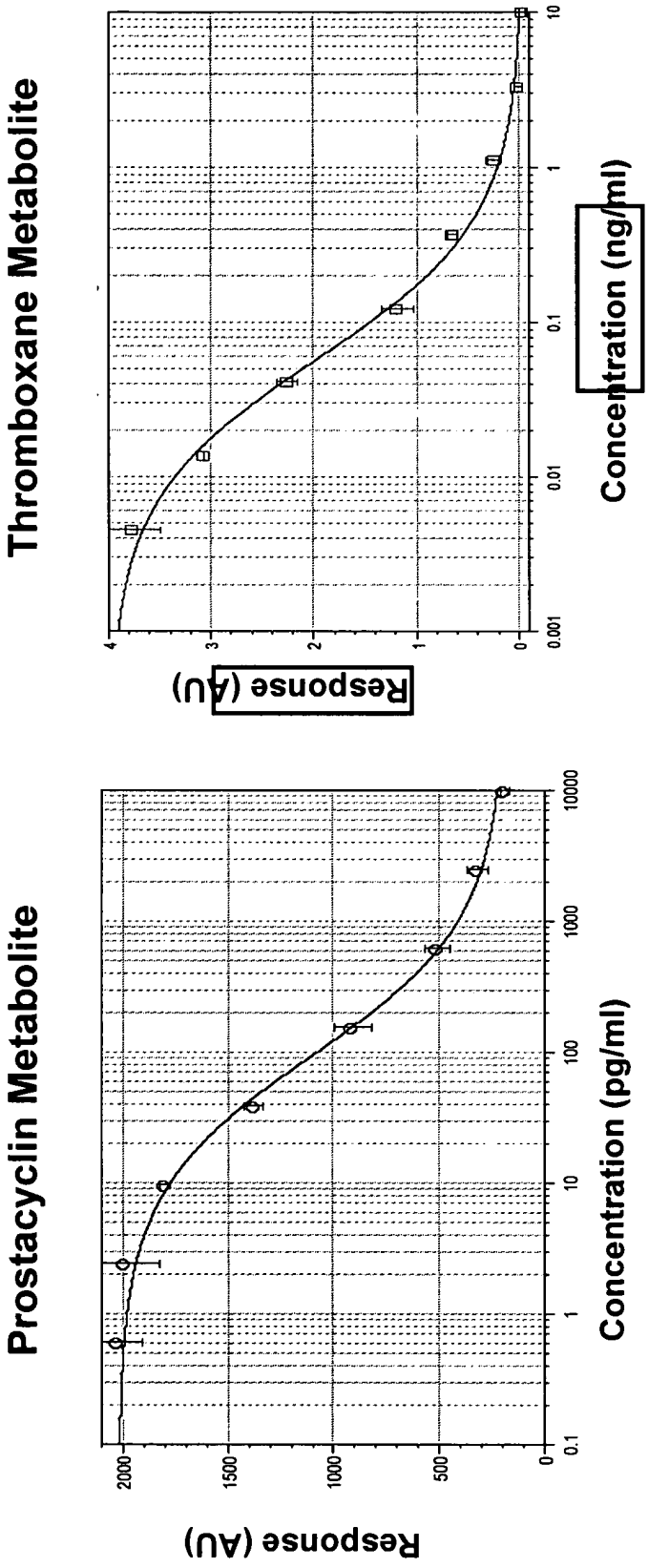


FIG. 11

Cartridge

- 5cm x 7.5cm x 7mm
  - Functionality designed into fluid layer
  - Reagents self contained
  - Access ports fixed

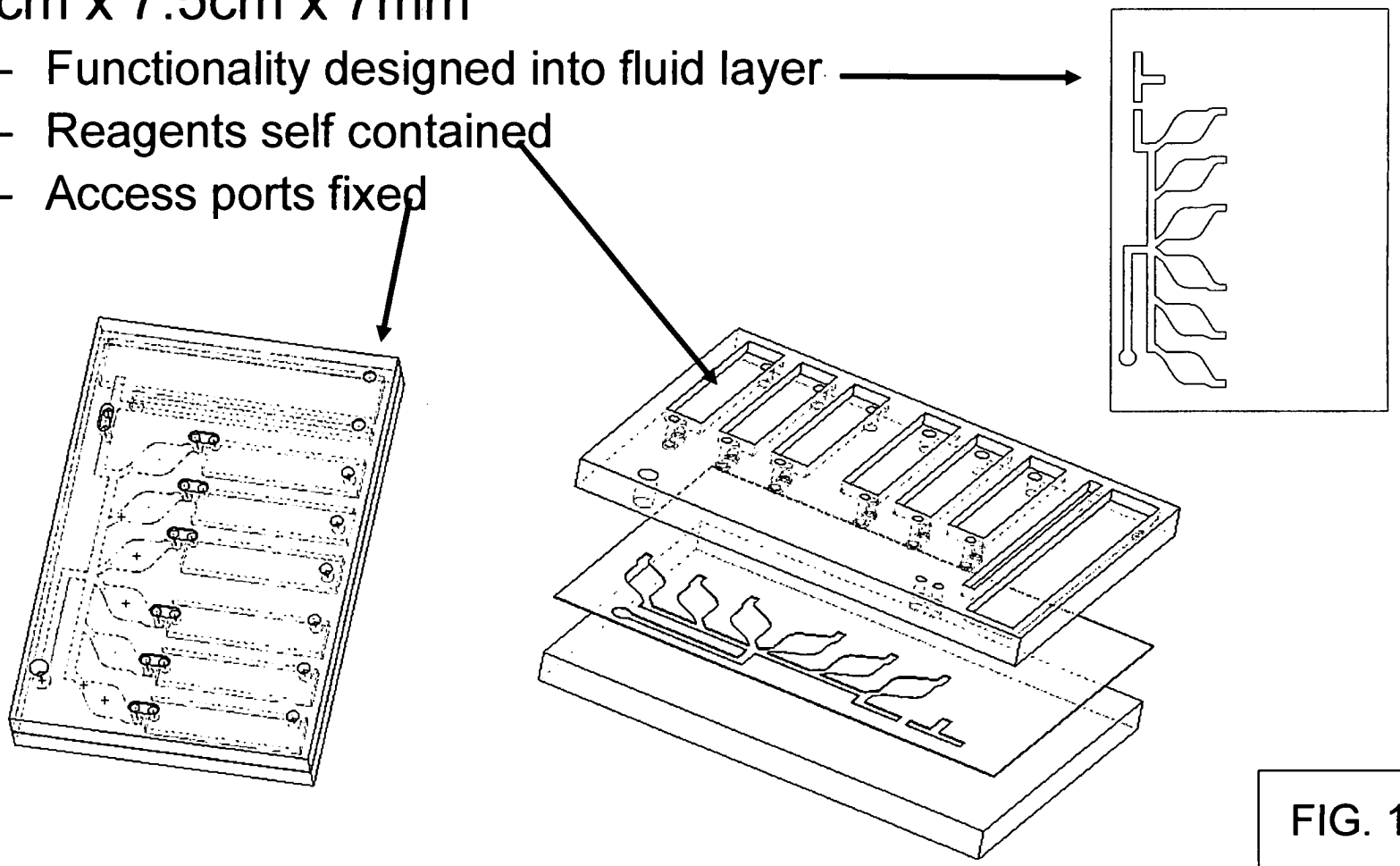
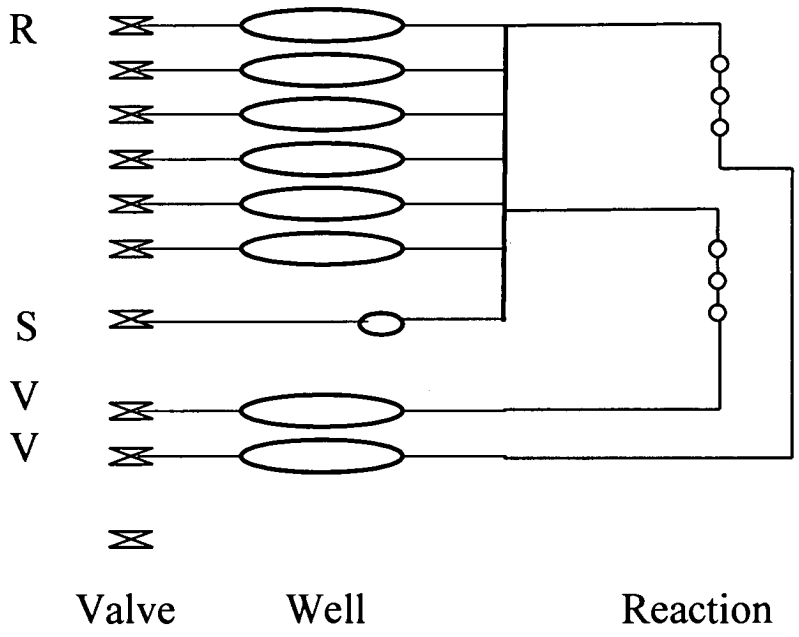


FIG. 12

Cartridge Fluid Flow Diagram



6 reagent wells
1 Sample well
2 waste wells

Each valve controlled individually

V connected vacuum

S sample port covered

R reagent ports

FIG. 13

PC Card Block Diagram

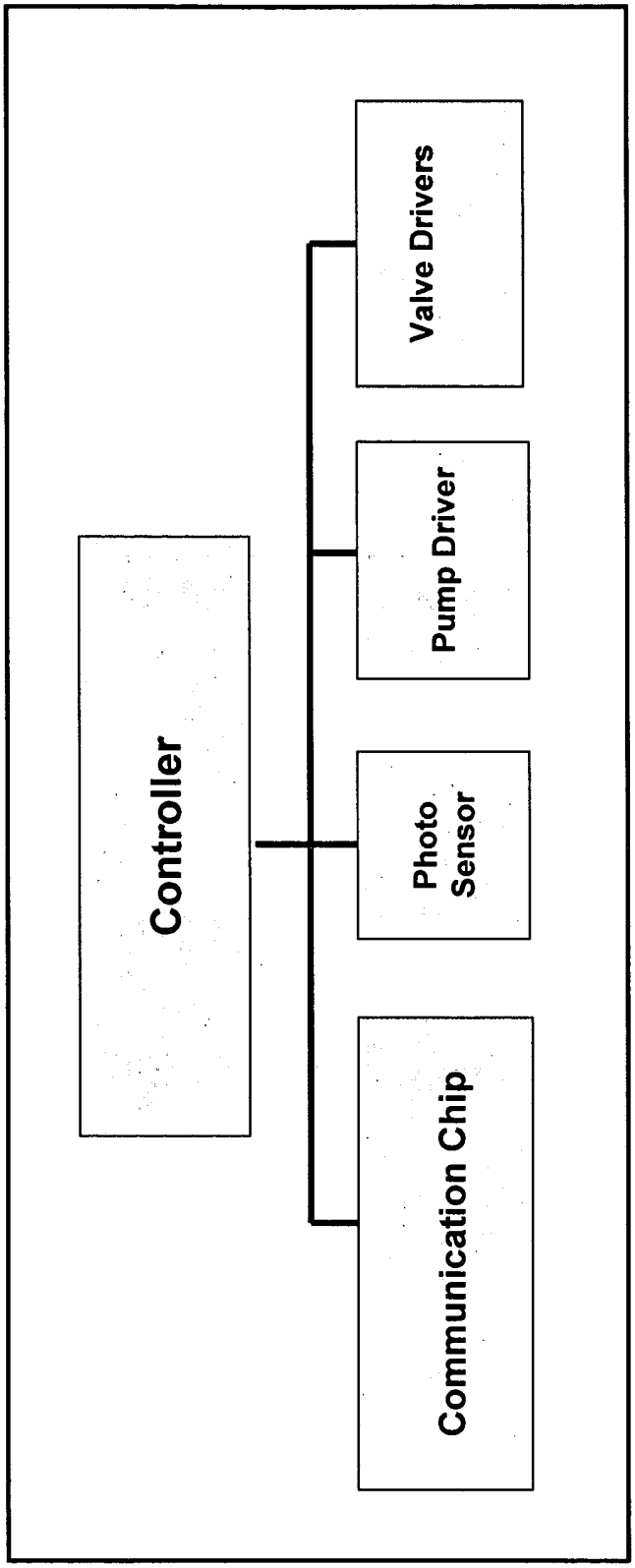


FIG. 14

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

05/11/2005 DEMMANU1 00000055 500417 60678801
01 FC:2005 100.00 DA

PTO-1556
(5/87)

*U.S. Government Printing Office: 2002 — 469-267/69033

EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
60/705,489	08/05/2005	Ian Gibbons	035738-0016

CONFIRMATION NO. 7487

OC000000019010261

20277
MCDERMOTT WILL & EMERY LLP
600 13TH STREET, N.W.
WASHINGTON, DC 20005-3096

Date Mailed: 05/30/2006

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/12/2006.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

MBeyene

MELKAM BEYENE
PTOSS (703) 305-3006

OFFICE COPY

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
60/705,489	08/05/2005	Ian Gibbons	30696-703.101

CONFIRMATION NO. 7487

OC000000019010272

021971
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

Date Mailed: 05/30/2006

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/12/2006.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

MELKAM BEYENE
PTOSS (703) 305-3006

OFFICE COPY

ASSIGNMENT OF APPLICATION	Docket Number 30696-703.101

Whereas, the undersigned:

1. Gibbons, Ian
   Portola Valley, CA 94028
2. Wang, Chengwang
   Mountain View, CA 94043
3. Roy, Shaunak
   San Mateo, CA 94403
4. Holmes, Elizabeth
   Palo Alto, CA 94301

hereinafter termed "Inventors", have invented certain new and useful improvements in

METHODS FOR MINIMIZING CALIBRATION ERRORS FOR ASSAYS PERFORMED IN DISPOSABLE ANALYTICAL SYSTEMS

☒ for which an application for United States Patent was filed on August 5, 2005, Application No. 60/705,489.
☐ for which a United States Patent issued on ___, U.S. Patent No. ___.

WHEREAS, Theranos, Inc., a corporation of the State of Delaware, having a place of business at 1430 O'Brien Drive, Suite H, Menlo Park, CA 94025, (hereinafter termed "Assignee"), is desirous of acquiring the entire right, title and interest in and to said application and the invention disclosed therein, and in and to all embodiments of the invention, heretofore conceived, made or discovered jointly or severally by said Inventors (all collectively hereinafter termed "said invention"), and in and to any and all patents, inventor's certificates and other forms of protection (hereinafter termed "patents") thereon granted in the United States and foreign countries.

NOW, THEREFORE, in consideration of good and valuable consideration acknowledged by said Inventors to have been received in full from said Assignee:

1. Said Inventors do hereby sell, assign, transfer and convey unto said Assignee the entire right, title and interest (a) in and to said application and said invention; (b) in and to all rights to apply for foreign patents on said invention pursuant to the International Convention for the Protection of Industrial Property or otherwise; (c) in and to any and all applications filed and any and all patents granted on said invention in the United States or any foreign country, including each and every application filed and each and every patent granted on any application which is a divisional, substitution, continuation, or continuation-in-part of any of said applications; and (d) in and to each and every reissue or extensions of any of said patents.

2. Said Inventors hereby jointly and severally covenant and agree to cooperate with said Assignee to enable said Assignee to enjoy to the fullest extent the right, title and interest herein conveyed in the United States and foreign countries. Such cooperation by said Inventors shall include prompt production of pertinent facts and documents, giving of testimony, execution of petitions, oaths, specifications, declarations or other papers, and other assistance all to the extent deemed necessary or desirable by said Assignee (a) for perfecting in said Assignee the right, title and interest herein conveyed; (b) for prosecuting any of said applications; (c) for filing and prosecuting substitute, divisional, continuing or additional applications covering said invention; (d) for filing and prosecuting applications for reissuance of any said patents; (e) for interference or other priority proceedings involving said invention; and (f) for legal proceedings involving said invention and any applications therefor and any patents granted thereon, including without limitation reissues and reexaminations, opposition proceedings, cancellation proceedings, priority contests, public use proceedings, infringement actions and court actions; provided, however, that the expense incurred by said Inventors in providing such cooperation shall be paid for by said Assignee.

3. The terms and covenants of this assignment shall inure to the benefit of said Assignee, its successors, assigns and other legal representatives, and shall be binding upon said Inventors, their respective heirs, legal representatives and assigns.

4. Said Inventors hereby jointly and severally warrant and represent that they have not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

IN WITNESS WHEREOF, said Inventors have executed and delivered this instrument to said Assignee as of the dates written below:

Date: 04/25/06 — Ian Gibbons

Date: 4/25/2006 — Chengwang Wang

Date: 04/28/06 — Shaunak Roy

Date: April 19, 2006 — Elizabeth Holmes

Practitioner's Docket No.: 30696-703.101 ***PATENT***

POWER OF ATTORNEY BY ASSIGNEE TO EXCLUSION OF INVENTOR UNDER 37 C.F.R. § 3.71 WITH REVOCATION OF PRIOR POWERS

The undersigned ASSIGNEE of the entire interest in:

☐ U.S. Patent No.
☒ U.S. application no. 60/705,489, filed on August 5, 2005

hereby appoints all Wilson Sonsini Goodrich & Rosati attorneys registered to practice before the United States Patent and Trademark Office, as associated with:

Customer No. 021971

to prosecute this application and transact all business in the United States Patent and Trademark Office in connection therewith and hereby revokes all prior powers of attorney; said appointment to be to the exclusion of the inventors and the inventors' attorneys in accordance with the provisions of 37 C.F.R. § 3.71.

The following evidentiary documents establish a chain of title from the original owner to the Assignee:

(complete one of the following)

☒ a copy of an Assignment attached hereto, which Assignment has been (or is herewith) forwarded to the Patent and Trademark Office for recording; or

☐ the Assignment recorded on ___ at reel ___, frames ___-___.

Pursuant to 37 C.F.R. § 3.73(b) the undersigned Assignee hereby states that evidentiary documents have been reviewed and hereby certifies that, to the best of ASSIGNEE's knowledge and belief, title is in the identified ASSIGNEE.

CHANGE OF CORRESPONDENCE ADDRESS

Direct all correspondence and telephone calls to:

Name	Karen K. Wong, Ph.D. J.D.					
Address	Wilson Sonsini Goodrich and Rosati					
Address	650 Page Mill Road					
City	Palo Alto	State	CA	Zip	94304	Customer No.: 021971
Country	USA	Telephone	(650) 493-9300	Fax	(650) 493-6811	

ASSIGNEE: Theranos, Inc.

Name: Elizabeth Holmes
Print

Signature

Title: President and CEO

Date: April 25, 2006.

Electronic Acknowledgement Receipt

EFS ID:	1046005
Application Number:	60705489
Confirmation Number:	7487
Title of Invention:	Methods for minimizing calibration errors for assays performed in disposable analytical systems
First Named Inventor:	Ian Gibbons
Customer Number:	20277
Filer:	Vernon A. Norviel/cathy bachmann/VN/KW/CB
Filer Authorized By:	Vernon A. Norviel
Attorney Docket Number:	035738-0016
Receipt Date:	12-MAY-2006
Filing Date:	05-AUG-2005
Time Stamp:	14:39:56
Application Type:	Provisional
International Application Number:	

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)	Multi Part	Pages
1	Power of Attorney (may include Associate POA)	30696-703-101POA.pdf	118688	no	2

Warnings:	
Information:	
Total Files Size (in bytes):	118688

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Express Mail Label No.				Docket Number	035738-0016
INVENTOR(s)/APPLICANT					
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (City and Either State or Foreign Country)		
GIBBONS	**Ian**		**Portola Valley, CA**		
WANG	**Cheng**		**Mountain View, CA**		
ROY	**Shaunak**		**San Mateo, CA**		
HOLMES	**Elizabeth**		**Palo Alto, CA**		
Additional inventors are being named on the separately numbered sheets attached hereto.					
TITLE OF THE INVENTION (500 characters max)					
METHODS FOR MINIMIZING CALIBRATION ERRORS FOR ASSAYS PERFORMED IN DISPOSABLE ANALYTICAL SYSTEMS					
CORRESPONDENCE ADDRESS					
McDERMOTT WILL & EMERY LLP 600 13th Street, N.W. Washington, D. C. 20005-3096 202.756.8000					
STATE	Washington, D. C.	**ZIP CODE**	20005-3096	**COUNTRY**	USA

ENCLOSED APPLICATION PARTS ***(check all that apply)***

☒ **Specification** ***Number of pages*** **[27]** ☒ **Small Entity Statement**

☒ **Drawings** ***Number of sheets*** **[12]** ☐ Other (specify): _____

Application Size Fee: If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CAR 1.16(s).

METHOD OF PAYMENT OF APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ **Applicant claims small entity status. See 37 CFR 1.27.** **TOTAL FEE ($)**

☐ A check or money order is enclosed to cover the filing fee and application size fee (if applicable). **$100.00**

☒ **The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number: 500417.**

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ **No.**

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

McDERMOTT WILL & EMERY LLP

Thomas A. Haag, Ph.D., Esq.
Registration No. 47,621

Please recognize our Customer No. 20277 as our correspondence address.

600 13th Street, N.W.
Washington, DC 20005-3096
Phone: 202.756.8000 TAH:gav
Facsimile: 202.756.8087

Date: August 5, 2005

METHODS FOR MINIMIZING CALIBRATION ERRORS FOR ASSAYS PERFORMED IN DISPOSABLE ANALYTICAL SYSTEMS

Inventors

Ian Gibbons, 831 La Mesa Drive, Portola Valley, CA 94028. Citizen of the UK.

Cheng Wang, 100 N Whisman Road Apt 3123, Mountain View, CA 94043. Citizen of Hong Kong SAR.

Shaunak Roy, 3222 Glendora Avenue #106, San Mateo, CA 94403. Citizen of USA.

Elizabeth Holmes, 325 Channing #118, Palo Alto, CA 94301. Citizen of USA.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] This invention relates generally to minimizing errors in assays for measuring analyte concentration due to incorrect calibration. More particularly, the invention relates to minimizing errors in assays performed in disposable systems, particularly those using immunoassays.

[0002] 2. Description of the Related Art

[0003] Assays, particularly immunoassays, of patient samples, require careful, precise calibration using data (a) gathered in parallel with the sample measurement by measuring known standards (calibrators) using the same assay protocol and reagents, or (b) provided by a manufacturer that are specific to a particular lot of reagents and assay conditions. Generally, such manufacturer provided calibration data are associated with strict temperature and other assay related conditions. Such calibration information is critical in determining the relationship between the response (output) from the assay system and the analyte concentration. Errors due to mis-calibration of distributed assay systems, especially in the case of immunoassays and particularly in the case of immunoassays that do not use "excess" reagents (such as competitive immunoassays and the so-called two-step assay described in U.S. Ser. No. 60/678,801, which is incorporated here in its entirety), could lead to significant errors in determining the concentration of an analyte of interest .

[0004] Immunoassays have a characteristic response similar in form to the well-known Scatchard binding isotherm (Bound/Maximum Bound (B/B0) = Ligand Conc./(K + Ligand Conc.) where B is the amount of the labeled analyte bound to a solid phase when analyte is present, B0 is the amount bound when no analyte is present and K is the dissociation constant. The mathematical form of such assay responses is hyperbolic.

[0005] Results of immunoassays of the types described above are typically analyzed using the so-called (ln-logit) or (log-logit) functions, in which the assay label (in the case of the two-step process, alkaline phosphatase-labeled analyte) bound to a solid phase when analyte is present in the assay ("B") is compared with the amount bound when no analyte is present ("B0)" to provide the ratio B/B0. Then, the "logit" function (logit = Log[(B/B0)/(1 – B/B0)]) is plotted against Log (Analyte Conc.) giving a straight line. (Natural logarithms can also be used instead of logarithms to base 10). The slope and intercept of this plot can be used to derive simple equations that permit one to calculate (a) assay signal as a function of analyte concentration, or (b) analyte concentration as a function of assay signal. An example of such analysis is shown in Fig. 1 using Thromboxane as the analyte of interest. The best fit to the data is given by Equation 1:

Signal = (A-D)/(1 + (Analyte conc./C)^B) + D [Equation 1]

where,
A is the signal at zero analyte concentration;
D is the signal at infinite analyte concentration;
C is the analyte concentration reached at a signal level half way between A and D; and
B is a shape parameter.

The relationship between analyte concentration and signal is given by:

Analyte conc. = C*((((A - D)/(Signal - D) - 1)^(1/B)) [Equation 2]

where A, B, C and D are identical to the parameters used in Equation 1.

[0006] Using these same equations it is possible to compute errors that occur from mis-calibration. (The Analyte Conc. function given above (Equation 2) is differentiated with respect to each potential variable (A, B, C, D and Signal). Estimates of the difference between the ideal

value of the variable and the actual value in the system are used as Δ values in the calculation (Δ(conc.) = (d(Conc.)/d(Param.))* Δ Param).

[0007] As can be understood, errors in calibration are reflected in erroneous values of A, B, C and D. Each of these parameters is influenced by a different factor. Temperature effects on calibration of immunoassays will have the strongest impact on the A, C and D parameters of the ln-logit calibration. It is likely that temperature effects will have minimal impact on the shape parameter, B.

[0008] The measured signal, which in turn is used to determine the concentration of the analyte(s) of interest, is biased by one or more of the following components involved in the measuring the signal in a disposable assay system:

a. Instrument Characteristics

1. Optics used in the instrument for signal measurement
2. Temperature control
3. Most chemical processes are highly temperature sensitive. In the present context, these include enzyme reactions, and equilibrium between antigens and antibodies.
4. Timing of assay steps
5. Calibration relative to an "ideal" instrument
6. Particularly important for distributed systems which cannot easily be re-calibrated in the field.

b. Variations in the Disposable

1. Dimensions
2. Volume of the assay chamber and its shape

ii. Fluid movement

iii. Timing and uniformity of fluid movement

iv. Efficiency in mixing (most assay methods used in disposables and employ microfluidics would involve some mixing)

c. Variations in the Reagents

1. Reagent quantity
2. Reagent dissolution (if it is in dry form)
3. Changes in activity of reagents following manufacture (instability). This is particularly important for "distributed systems" where the disposable useful life is typically determined by reagent stability. If the reagents have lost, for example, 20% of their activity, which is quite common, but if they can be used without significantly compromising assay performance, the shelf-life of many expensive disposables could be extended several fold and severe constraints on disposable storage (refrigeration and the like) can be relaxed.

d. Factory calibration parameters

1. When calibration is performed at the factory, even small errors in the estimation of the calibration parameters result in error in the derived analyte concentration.

[0009] The magnitudes of these calibration errors and consequently errors introduced in estimating analyte concentrations can be quite significant. FIG. 1 shows the dose-response data for a two-step assay for Thromboxane. The top curve (Logit.test) in FIG. 2 shows a typical (ln-logit) assay response. When we adjust the level of the highest signal (A) and the lowest signal (D), shown as "Shift zero signal" and "Shift 100% signal", respectively, the curves shift as seen in FIG. 2. The corresponding computed values of error in the concentration that would be calculated from Equation 2 were large (> 20 % across the entire range of the assay) as shown in FIG. 3. In FIG. 2, the signal is normalized by subtracting the D value from the signal and dividing the difference by (A-D):(Signal – D)/(A – D). This yields what is usually described as B/B_0 (the ratio of bound label at a given analyte concentration to that at zero analyte level). The ln-logit function was modified by adding 10% of (A - D) to D or subtracting 10% of (A – D) from A before recalculating the normalized signals (corresponding to two types of significant

calibration error (shifting the value of A or D respectively). At signal levels intermediate between A and D the change made was adjusted to by 10%*(Original signal – D)/(A-D). FIG. 3 shows that when modifications of only 1%*(A - D) were made, and concentration of the analyte was computed, errors in concentration were still significant at certain parts of the analyte concentration range.

[0010] In a laboratory setting, errors in measuring biochemical parameters of blood and other bodily fluids due to calibration errors are dealt with using many known compensation mechanisms. One of the simplest techniques is to add a known quantity of a trace amount of a radiolabeled analyte and construct a calibration curve based on those readings. Other methods include adding a known amount of a standard to the analyte solution that needs to be analyzed. However, such techniques are impractical in a disposable, handheld system for analysis, without particular adaptation of those techniques for dealing with small sample volumes, lack of large amounts of other solutions (such as buffers), and ability to exercise precise controls over the volumes of the samples and their dilutions.

[0011] In disposable systems, particularly in those where the sample acquisition is done by the patient/consumer, measurement errors creep in for various reasons. These significant errors, due to the patient handling the sample, could be due to the sample collection methods. The patient may not collect the correct volume of the sample at the appropriate time, or may not handle the collected sample in an appropriate manner, compromising the sample integrity. It will be highly beneficial to have a disposable system, wherein the patient controls the initial sample collection and handling, have means for minimizing the consequences of such errors by either alerting the patient to repeat the test or use calibration mechanisms to compensate for such errors.

[0012] Hence, there is a significant need for methods that would improve the calibration in hand held or disposable assay units; particularly in those units where the sample and reagent volumes are in the microliter and nanoliter ranges; maintaining controlled temperatures are impractical; the sample is not "clean" where errors are introduced due to the presence of

interfering substances, such as hematocrit, etc.; or it is difficult to maintain the desired conditions (temperature, reagent quality, etc.), including the appropriate sample volume and handling in the field or in the hands of the consumer.

SUMMARY OF THE INVENTION

[0013] The present invention overcomes the limitations of the prior art by providing methods for improving the accuracy and reliability of calibration procedures in disposable systems that are capable of analyzing analytes and markers in microliter quantities of blood or plasma samples.

[0014] In one aspect, the invention is a method for correcting errors in the calibration parameters in a self-contained disposable analytical system comprising measuring within the system one or more parameters of a calibration curve, comparing those parameter values with the same parameters that were established when the lot of disposables was calibrated by the manufacturer, and then adjusting a signal output by the ratio of the parameter values determined within the system to the parameter values established by the manufacturer.

[0015] In another aspect of the invention, the manufacturer's parameter values that are to be used in a calibration curve to scale a signal to determine an analyte concentration is replaced by the parameter values measured within the system.

[0016] In yet another embodiment of the invention, the parameter values will be measured in a buffer sample for each analyte.

[0017] In another aspect, this invention is a method of improving the accuracy of calibration in a self-contained disposable analytical system by measuring a first signal in an original sample containing a known quantity of an analyte, measuring a second signal after spiking the original sample with a known quantity of the analyte, plotting the difference between the first and second signals against a target value, where the target value is the signal expected for the known quantity of the analyte, and arriving at a best fit of parameters by minimizing the sum of the square of the differences between target and calculated analyte values.

[0018] Another embodiment of this invention is a method of improving the accuracy of calibration parameters in a self-contained, disposable analytical system by first calculating in a sample containing an unknown concentration (x1) of an analyte using standard calibration parameters, spiking the unknown sample with a known concentration of the analyte (x_{spike}), determining known concentration in the spiked sample using the standard calibration parameters (x2), and correcting the unknown concentration by a factor, where the factor is the ratio between the difference between x2 and x1 and x_{spike}.

[0019] In one embodiment of this invention, the accuracy of the measurement in a disposable system used by a patient is ensured by using calibration methods carried by wireless or cellular communication with a server or computer at network headquarters, wherein the server checks for the integrity and volume of the sample collected by the patient and placed in the disposable assay system, and appropriate corrective action is taken by the server. One appropriate action could be instruction to the patient to provide a new sample; another appropriate action could be to take into consideration the deviation in sample volume from the expected sample volume, and hence the variation in the expected signal strength.

[0020] In another aspect of this invention, errors due to malfunctions in the disposable system are sensed by onboard sensors on the handheld and communicated to the back-end servers or computers at network headquarters and if a patient needs feedback, such feedback is displayed on the handheld and/or appropriate corrective calibration values are received and incorporated into the assays.

[0021] Other aspects of the invention include devices and systems corresponding to the methods above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] The invention has other advantages and features which will be more readily apparent from the following detailed description of the invention and the appended claims, when taken in conjunction with the accompanying drawings, in which:

[0023] FIG. 1 is a plot of dose-response data in a typical two-step assay.

[0024] FIG. 2 shows dose responses computed with and without errors in calibration parameters.

[0025] FIG. 3 shows computed concentration errors produced by 1% mis-estimation of A and D calibration values.

[0026] FIG. 4 shows calibration using a "differential" approach.

[0027] FIG. 5 shows verification of calibration using the "1-point spike" method (log scale)

[0028] FIG. 6 shows verification of calibration using the "1-point spike" method (linear scale).

[0029] FIG. 6a shows using spike recovery to eliminate calibration errors of the "C" parameter.

[0030] FIG. 7 shows dose-response of assays calibrated against a plasma sample with a very low TxB2 concentration.

[0031] FIG. 8 shows a method for calculating difference in concentration between two samples.

[0032] FIG. 9 illustrates analyte assay in plasma samples.

[0033] FIG. 10 shows the time course of change in the calibration values as a function of time.

[0034] FIG. 11 shows the impact of change in calibration parameter A on assay calibration.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0035] Although the detailed description contains many specifics, these should not be construed as limiting the scope of the invention but merely as illustrating different examples and aspects of the invention. It should be appreciated that the scope of the invention includes other embodiments not discussed in detail above. Various other modifications, changes and variations which will be apparent to those skilled in the art may be made in the arrangement, operation and details of the method and apparatus of the present invention disclosed herein without departing from the spirit and scope of the invention as described here.

[0036] Conventionally, while assaying samples in a laboratory, a calibration exercise is performed in parallel with the measuring analyte concentration(s) of interest in the body fluid (blood, plasma, etc.)--sample assays. This is impractical in a self-contained, disposable assay system that is intended to be compact and inexpensive. In one embodiment of our invention to address the calibration challenges while assaying analytes using a disposable system, parameters A, or preferably A and D, of Equation 1 are measured within the device as opposed to using manufacturer's values or using an external device. This value is compared with the parameter values estimated when the lot of disposables is calibrated by the manufacturer. Signal results are then adjusted using the following equation:

$Signal_{adjusted} = Signal*(A_{factory\ calibration}/A_{measured\ within\ the\ assay})$ and the original calibration equation (Equation 1) is used to calculate the analyte concentration(s). Alternatively A and D values measured at the time of assay (in the disposable system) are substituted for the original A and D values obtained at factory calibration. Typically the (A/D) calibration measurement would be made in a buffer sample. It could be made for:

1. each analyte (in a multiple analyte assay device), which is a preferred method; or
2. one analyte only, if each assay responds similarly to the various factors that change the calibration parameters.

This approach for improving the accuracy of the computed concentrations can account for the changes in the calibration parameters due to:

(a) Temperature changes during the assay

It is particularly desirable not to have to control temperature during an assay in a self-contained, disposable system, because of the power requirements. This is especially true for assays requiring long incubation times. In a hand held or disposable device, it will be impractical to tightly control the temperature at which the assay is run. Hence, a method that can compensate for changes in temperature, such as the one described above, can be quite useful.

(b) Instability of the enzyme and substrate reagents on storage

(c) Differences in reagent dissolution (for dry reagents) and recovery of activity when sample and/or solution phase reagents are added to dry reagents

(d) Variations of certain disposable dimensions, and

(e) Variations of the performance of the measurement instrument (from time-to-time and instrument-to-instrument)

[0037] In another embodiment of this invention, the calibration parameters of Equation 1 are corrected using differential calibration. The following example illustrates this approach using Thromboxane B2 as the analyte whose concentration is to be determined in plasma.

[0038] Thromboxane B2 (TxB2) (1.25 mg) was dissolved in a mixture of dimethylsulfoxide (342 µl) and water (342 µl). To this 5 µl of a solution of 1-(3-(dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride in water (0.1 g/ml) and 10 µl of a solution of n-hydroxy-succinimide in water (0.1 g/ml) were added. After 1 hour at room temperature the resulting NHS-ester of TxB2 was used in the preparation of TxB2 labeled with alkaline phosphatase (described below) without further purification. Alkaline phosphatase (bovine intestine, Sigma-Aldrich) was dissolved in phosphate-buffered saline at 1 mg/ml. To 1 ml of this solution 120 µl of the NHS-ester of TxB2 was added and the mixture allowed to react for 1 hour at room temperature. The enzyme-TxB2 conjugate was then purified overnight by dialysis against tris-buffered saline containing $MgCl_2$.

[0039] "Two step" enzyme immunoassay for TxB2

[0040] Samples and mouse monoclonal anti-TxB2 (15 µl of Cayman Chemical Kit Catalog number 10005065, appropriately diluted into Assay Designs buffer) were added to 384-well plates to which anti-Mouse IgG had been immobilized ((Becton Dickenson 356177)). The sample was 30 µl of plasma diluted 1:4 with assay buffer (Assay Designs Correlate-CLIA™ kit 910-002) and supplemented with known concentrations of TxB2. Other types of sample (for example TxB2 dissolved in assay buffer) can be substituted.

[0041] Plates were covered to prevent evaporation and incubated at room temperature with gentle mixing (100 rpm) on an orbital shaker for 12 hours. The contents of the wells were then removed by aspiration. Thromboxane-labeled with alkaline phosphatase (25 µl diluted 1: 1500 with assay buffer) was added and incubated at room temperature for 2 minutes. The contents of the wells were removed by aspiration and wells washed thrice with 100 µl wash buffer (from the Assay Designs Kit 910-002).

[0042] Enzyme bound to the wells was then measured by addition of 40 µl Lumiphos™ 530 substrate solution which contains (4-methoxy-4-(3-phosphate-phenyl-spiro-[1,2-dioxetane-3,2'-adamantane])). Incubation was allowed to proceed for 1 hour with orbital mixing and the luminescent product measured in a Molecular Devices MD5 Spectrometer (0.5 second integration time).

[0043] FIG. 1 shows the typical assay dose-response data for a two-step assay for TxB2. Using Equation 1, the parameters A, B, C and D are fitted to the curve shown in FIG. 1. As described above, even small changes in values of parameters A and D have a significant impact on the measured concentration. Hence, any errors in computing A and D are magnified in the estimated analyte (TxB2) concentration. This concept is illustrated in FIGs. 2 and 3, where even a 1% change in (A-D) resulted in significant errors in estimating TxB2 concentrations in the samples. In this FIG. 2, the signal is normalized by subtracting the D value and dividing the

difference by (A-D) viz: (Signal – D)/(A – D). This gives what is usually described as B/B0 (the ratio of bound label at a given analyte concentration to that at zero analyte level). The (ln-logit) function was modified by adding 10% of (A - D) to D or subtracting 10% of (A – D) from A before recalculating the normalized signals (corresponding to two types of significant calibration error (shifting the value of A or D respectively). At signal levels intermediate between A and D the change made was adjusted to by 10%*(Original signal – D)/(A-D). FIG. 3 shows the computed errors in estimating the analyte concentrations for a 1% error in estimating A and D. As can be seen, for the low analyte concentrations, the errors are very pronounced even for small errors in the calibration parameters A and D.

[0044] FIGs. 4-7 illustrate another embodiment of this invention, where the sample containing an unknown concentration of an analyte is spiked with a known concentration of the analyte to minimize calibration errors. Spiking can be achieved by many methods. E.g., by incorporating analyte in known, precise quantities to (typically) the assay well during manufacture of the disposable. Other locations for spike analyte are also possible. Separate spike wells could be accommodated in the disposable cartridge that is described in the copending applications filed by the applicant. FIG. 4 shows calibration using differences between signal response between unspiked and spiked samples. Amount of the spiked material is indicated by x2 and the original (endogenous concentration in the sample) is denoted as original concentration or x1 (pg/ml). The difference in signal between unspiked and spiked sample is plotted against the signal for the original concentration for various amounts of known amount of analyte (spike) introduced into the sample. The (ln-logit) parameters (for the top curve in FIG. 4) are shown in Table 1.

[0045] Table 1: Original Calibration Parameters for Data Shown in FIG.4

A	3.37E+04
B	1.01E+00
C	2.10E+02
D	3.56E+03

The data shown in the top curve in FIG. 4 were used in a recalibration exercise by calibrating against the difference in signal for each original concentration level and each level spiked with 200 pg/ml analyte. Equation 3, which is shown below, was empirically derived and found to be useful in calculating the original endogenous concentration of analyte. The best-fit parameter values in Table 2 were computed by minimization of the sum of the square of the differences between target and calculated analyte values (using the "Solver" function in Excel).

Conc. = C*((A - D)/((Signal - D)^(1/B)) + E (Equation 3)

Table 2: Calculated Parameter Values for 1-point Spike Calibration

A	1.20E+02
B	1.996189
C	292.7824
D	-0.14393
E	-287.931

This calibration was verified as shown in FIG. 5 (log scale) and FIG. 6 (linear scale). Note the regression equation was calculated for data in linear form. The formula gave almost perfect results.

[0046] The results of another embodiment of this invention are shown in FIG. 7, where the extent of the recovery of the spike signal is used to correct for the concentration of the value of the unspiked sample. The method has the advantage that changes in the parameter C in the (ln-logit) equation due to reagent instability etc. is accounted for. The method involves the following steps:

1. Calculate x1 (endogenous conc.), and x2 (spike conc.) using original calibration
2. Calculate recovery of spike as % (x2 - x1)/spike *(Equation 4)*
3. Correct x1 by recovery factor: (x1*100/Spike recovery) *(Equation 5)*

This was tested with the calibration curve shown in FIG. 4 and the original calibration parameters of Table 1. As shown in Table 3, it was possible to use spike concentration values from 100 - 500 pg/ml and C values that varied from 500 to 50 such that the actual signals corresponding to the modified C values were changed very significantly from what had been the case with the original C value and the spike recovery (calculated with the original C value ranged from 42 – 420 % respectively, yet the recovery of the unspiked sample (once corrected for the recovery of the spike) was 100% over the entire calibration range. This effect is graphically illustrated in FIG. 6a, where the C parameter is varied between 50 and 500 (a ten fold range), but the corrected values for the analyte concentration (x1) accurately reflects the expected analtye concentration.

Table 3: Effects of changes in the C parameter on spike and original analyte recovery at two original concentration levels

C	x1	S (x1)	x2	S (x1+x2)	x2 recovery	x1 recovery
	pg/ml		pg/ml		%	%
500	100	2.88E+04	500	1.73E+06	42	100
210	100	2.40E+04	500	1.13E+04	100	100
50	100	1.36E+04	500	5.83E+03	420	100
500	316	2.21E+04	500	1.50E+04	42	100
210	316	1.56E+04	500	9.66E+03	100	100
50	316	7.61E+03	500	5.25E+03	420	100
500	100	2.88E+04	200	2.25E+04	42	100
210	100	2.40E+04	200	1.60E+04	100	100
50	100	1.36E+04	200	7.80E+03	420	100
500	316	2.21E+04	200	1.84E+04	42	100
210	316	1.56E+04	200	1.22E+04	100	100
50	316	7.61E+03	200	6.16E+03	420	100

In Table 3, x1 is the endogenous concentration, and x2 is the spike concentration; S is the signal level corresponding to the designated analyte concentration; x2 recovery is the apparent recovery of x2 and x1 recovery is calculated (using Equation 5) after compensating for x2 recovery (using Equation 4).

[0047] It should be noted that the spike level has to be carefully chosen. The optimal level will be a compromise between the operating range of the assay and the likely range of

concentrations of samples. If it is too low, the change in signal caused by the spike will be too small to be reliably measured. If it is too high, the assay response will be too shallow to reliably measure the spike. The ideal spike level would change the measured signal by much more than the standard deviation in the signal. In the above example, the assay range had been adjusted to make measurements for sample with concentrations in the range 0 – 500 pg/ml and spikes of 200 – 1000 pg/ml would likely be useful.

[0048] Various guidelines for choosing spike levels can be followed:

1. Spikes should change the observed signal across the desired range by at least 10%.
2. Spikes should be in the same range as the anticipated mid range of sample concentrations.
3. Spikes should be less than about three times the original C value. (Note that the useful part of the dose-response is from about 0.2*C to 5* C.)

The following example illustrates the use of spike recovery to estimate the endogenous concentration of an analyte, in this case TxB2.

Estimation of Endogenous TxB2 Concentrations by Spike Recovery

Original data: Two citrated human plasma samples were analyzed by the two-step assay. Aliquots of the samples were also supplemented (spiked) with known concentrations of TxB2 prior to assay. Some samples were also supplemented with indomethacin (0.1 mM) and/or EDTA (5 mM). Samples were stored either flash-frozen then thawed or refrigerated unfrozen prior to assay. These procedures generated a set of samples with various original endogenous concentrations (storage and freezing and thawing tends to cause platelet activation and production of TxB2; indomethacin inhibits TxB2 production).

[0049] The results of the above experiment are shown in FIG. 7. Sample 5A was known to have a very low TxB2 concentration (estimated to be < 10 pg/ml). When the dose-response of the assay in sample 5 was used to calibrate the assay, we assumed this concentration to be zero. Dose responses for the other samples 4A, 4N, 5N were then plotted and it was observed that their response corresponded to higher concentrations of TxB2 and could be fitted to the 5N response

by moving each to the left (in the direction of lower concentration) by an amount corresponding to removing a certain fixed TxB2 concentration from each the known spike levels. In other words all the samples had responses that were almost identical in shape to that of sample 5N. When the curves fitted as closely as possibly to the A5 curve, the concentration of TxB2 notionally removed corresponds to the estimate of the TxB2 concentration in the sample.

[0050] The original data of FIG. 7 were represented in FIG. 8 by the best fit (ln-logit) approximation. The Solver function in Microsoft Excel was used to compute a value of TxB2 that caused the A5 response to approximate that of the sample N5. As can be seen, this generated a good fit and the computed value (471 pg/ml) is an estimate of the concentration difference between TxB2 levels in the two samples.

[0051] **Single Point Spike**

[0052] In another embodiment of our invention, with sufficiently precise data, a single-point spike could be used (all the points fit closely to the calibration curve, so any single point could have been used) as compared to a multi point spike that was illustrated in the earlier FIGs. 4-7. The following experiment illustrates this concept.

[0053] Two plasma samples were spiked to many levels of TxB2 and assayed by the two-step method. Assays were calibrated using buffer calibrators rather than plasma-based materials. Results are presented in FIG. 9. Plasma was analyzed as described earlier. Data in FIG. 9 are plotted on a log scale. The concentration of unspiked samples was calculated from the calibration and the concentration of spiked samples taken as "endogenous + spike." Results are plotted only for the spiked samples. As can be seen, there was good correlation between the calculated and known values over the range of about 50 – 10,000 pg/ml. When recovery was estimated for spikes in the range 40 - 2,500 pg/ml, the correlation was 99.7%.

[0054] Spike recovery method for correcting the calibration parameters are useful for compensating temperature effects on immunoassays in self-contained disposable analytical systems, also some times referred to as handheld analytical systems or assay systems. As is well known, instabilities in temperature during an assay introduce significant errors in the estimated analyte concentration. Temperature effects on calibration of immunoassays have the strongest impact on the A, C and D parameters of the (ln-logit) calibration. It is likely that the B (shape) parameter is minimally affected by temperature changes. As shown above, the spike recovery method can correct for errors introduced in the C parameter and hence could be an excellent approach for correcting temperature induced errors in computing the calibration parameters of the (ln-logit) equation. Similarly, normalizing signal levels to the zero analyte calibrator level, as described earlier, can compensate for errors in the A and D parameters, which are again negatively influenced by temperature changes.

[0055] Internal calibration and/or spike recovery means of calibration have significant advantages relative to conventional "factory-calibration" schemes. One obvious advantage is that two pieces of assay related information are used to compute the assay result rather than just one which will improve the reliability of the process. A second advantage is that this approach compensates, to a large extent, reagent instability. A third advantage is that several instrument, assay environment and procedural variables are compensated.

[0056] Other than temperature, other uncontrolled changes in system response can also negatively impact the computed A and D parameters. For example, FIG. 10 shows the time course of the signal generation during an assay. To correct for these errors, one embodiment of the claimed invention is to compare assay signals B in a disposable system with the B0 signal so to eliminate errors due to variation of the absolute value of assay signals due to uncontrolled changes in system response. This concept was verified by the following experiment.

[0057] A competitive immunoassay for TxB2 was set up using the protocol described in Assay Designs Product Literature for their corresponding Correlate-CLEIA kit (catalog 910-002).

An alkaline phosphatase conjugate was prepared as described earlier and was diluted 1:112,000 and substituted for the kit conjugate. A and D parameters are the calibration parameters used in the (log-logit) fit to the assay response. Best fit values were obtained at each time point. Note that at zero time the A and D parameters are not measured, but all signal values would be (are known to be) zero. The ratio D/A was multiplied by 1e6 so as to be presentable on the same scale. The A and D values when plotted against time vary significantly, particularly the A value (zero analyte). As seen from the straight line with practically zero slope, the scaled D/A remains constant over the time span.

[0058] The above experimental data were then analyzed by normalizing the assay signal (B) to signal at zero analyte concentration (B0). Using this normalized signal (B/B0), (log-logit) best fits were obtained for each time point and averaged. Concentrations of analyte were computed using these calibration parameters for each time. FIG. 11 shows the derived concentrations that were plotted against the A parameter derived for each individual time point. Each line corresponds to different analyte levels (pg/ml) ranging from 39 to 10,000 pg/ml. As can be seen from FIG. 11, although signal values changed by about 2-fold during the course of the experiment, the derived analyte concentration was essentially constant over the analyte concentration spanning a range of 39 to 10,000 pg/ml. The variation of calculated concentration was computed and found to average only 2.7 % over the calibration range of 39-625 pg/ml (which spans most of the range).

[0059] A calibration spike could be enabled by (1) adding analyte to the antibody (or other solid phase capture agent) during manufacturing, and then drying or (2) subsequent to adding analyte to the appropriate well during manufacturing (then drying), or (3) adding analyte to a portion of assay buffer which is then routed to the appropriate well. Methods 1 and 2 have a risk which is that the spiked analyte could be flushed from the well as sample or buffer enters. This may be handled in one of several ways such as (1) relying on the tightness of the antigen: antibody interaction for the brief time the well is subject to flowing sample or buffer (which exit

from the well), (2) careful management of liquid flow and placing the spike well as that most distal to the incoming liquid (last well to fill has the least flow through).

[0060] As noted earlier, errors in measuring analyte concentrations could also be due to variability in the pre-analysis phase. The primary cause of this type of errors is due to the patient collecting an incorrect volume of sample or where the sample integrity has been compromised. Errors due to incorrect sampling volume can by corrected by many means. One simple method is to measure the volume of the sample during a pre-processing step. If the measured volume is significantly different from the expected volume, the patient could be either instructed to provide a new sample. Alternatively, particularly in the case of infants or patients where collecting another sample will be inconvenient or impractical, the information that the volume is incorrect could be communicated to a back-end server that interfaces with the processor on the disposable or hand held unit. The analytical methods could be recalibrated to compensate for the change in the sample volume. The recalibration could be using any of the standard calibration techniques or the modifications to the calibration process, which have been described above.

[0061] The following is a description of a method of determining the accuracy of the volume of the sample located in the sample well of a handheld analytical system or the cassette as described in the co-pending U.S. App. Ser. No. 10/937,872. The sample well can be lined with conductive elements spaced apart at known separations—similar to the graduations on a measuring cylinder or jar. The location of each conductor will correspond to a specific sample volume. As fluid comes into contact with the conductor, the measured conductivity of that conductor would be markedly increased. By identifying the highest placed conductor that has undergone the conductivity change, one would be able to compute the volume of the sample in the sample well.

[0062] Alternatively, if the sample volume has to meet a minimum, a conductive element could be placed at the appropriate level in the well. When the cassette is introduced into the handheld (or the sample holder is introduced in the analytical system), thereby the patient has

indicated that she has completed the sampling process, and if the conductivity of the sensor remains at the baseline level, it could be easily concluded that the patient has not provided the required sample volume. The patient could be given the appropriate feedback such as replacing the sample or replenishing it. Alternatively, the back-end server or computer at the network headquarters could be informed of the issue and appropriate corrective measures taken. An alternative to the electrical sensing for the correct volume could be using known optical sensing means.

[0063] Sample integrity could be affected by many factors, some intrinsic to the patient and some that are extrinsic. Following are some of the sources of errors in sample integrity: (i) mixing of interstitial fluid with blood; (ii) variability in the hematocrit concentration; (iii) hemolysis; and (iv) activation of platelets and sample clotting.

[0064] Occasionally, interstitial fluid may leak from a finger-puncture wound and could mix with blood. Alternatively, if the patient had liquid on her hands due to washing prior to obtaining a blood sample, such liquid could also mix with blood plasma. Both fluids mentioned, above, interstitial fluid and wash liquid, contain no red cells and would mix with the blood plasma. When the amount of interstitial fluid is large so that the effective hematocrit is very low, the measured concentration of the external standard (fluorescein) would be low. This signal could be used to conclude that the sample is inappropriate for analysis and that it could lead to incorrect results. When blood is contaminated by water (which has low conductivity), it would be possible to detect this by measuring the conductivity of the fluid part of the sample (blood plasma has a characteristic high conductivity not subject to variation from day-to-day or individual-to-individual). If the measured conductivity of the sample is lower than the plasma conductivity, it is likely that the sample has been contaminated.

[0065] Errors could also be due to incorrect operation of the instrument and means of detecting and compensating those errors are described below. One source of error could be that the disposable is not properly accommodated in the handheld system. Having a sensor detect and

report the proper mating of the disposable in the handheld would be one means of avoiding this problem. Another source of errors is from the fluidic system, where there may be an issue with where the sample is applied in the sample well and the volume of the applied sample. This could again be addressed by the use of appropriate sensors which detect the application of a sample and report on the adequacy of the volume that is applied. Other fluidics related problems could be blocked channels, insufficient reagents, bubbles, etc., all of which again could be detected and reported by the use of appropriate sensors.

[0066] Any of these errors—incorrect volumes, improper mating of the cassette, operational issues with the fluidics system, etc.—could be sensed using sensors on the handheld. The error messages could be displayed on an LCD screen in the handheld using the processing power of the microchip on the handheld. Alternatively, the signals from the sensors could be communicated to the back-end server or computer at the network headquarters, which could then relay to the handheld the appropriate corrective action. Such action could be a message that would be communicated to the patient. This could be in the form of an audio, video or simple text message that the patient could receive on the handheld. Another action could be the back-end server transmitting corrections to the calibration parameters to compensate for any of the errors described above.

[0067] In yet another embodiment, the transmitter in the device is turned on to synchronize with the sever during the calibration phase of the device. The software allows reading of a barcode on the cartridge which determines the calibration protocols, language, and alignment of the valves and photodiodes in the system. If any of the signal transmitted by the sensor received doesn't match the expected value for that sensor signal, then the server transmits a pre-programmed reading based on each cartridge bar code to the LCD read-out display to (a) correct movement of the valves or proper insertion of blood into the cartridge via one of twelve actions pre-determined in the software protocols:

Error Code	Symbol	Problem	Action
Er1	Thermometer	Temperature out of range	Wait until Temp >10 or < 35 C
Er2	Blood drop	Blood sample too small	If detected w/in 15 minutes of first sample add more blood, other wise use new cartridge
Er3	Battery	Power disruption	Do not start test until power resumes
Er4	Bar code symbol	Cartridge expired	Run test on a non expired cartridge
Er5	Cartridge w a line through it	Cartridge already used	Run test on a new cartridge
Er6	Phone receiver	No Cell Phone coverage	Do not start test until in coverage area
Er7	Box w a line through it	Reader malfunction	Call Theranos
Er8	Bottle with a "C" in the label	Calibration overdue	Run Calibration standard, then run test

Data is transmitted between receivers to determine how much fluid to mix or how to recalibrate and the system responds successfully. Accordingly, the system displays confirmation of successful 1) calibration and 2) reading. or (b) display an error message to begin with a new cartridge or call a help line if the system and user are unable to correct any malfuctions.

[0068] Despite all the corrective actions, for many reasons, the measured values could still be erroneous. The actual analyte concentration could be well outside the expected range, and hence the calibration parameters used may be incorrect. Those values which are unlikely, impossible or inconsistent with prior data for a patient could be flagged and subjected to a software review. Those values that seem to be suspect can be communicated to the appropriate decision maker, such as the patient's physician.

METHODS FOR MINIMIZING CALIBRATION ERRORS FOR ASSAYS PERFORMED IN DISPOSABLE ANALYTICAL SYSTEMS

ABSTRACT OF THE DISCLOSURE

In a self-contained, disposable analytical assay system, internal calibration and/or spike recovery means of calibration have advantages relative to conventional "factory-calibration" schemes. Two pieces of assay related information are used to compute the assay result rather than just one which will improve the reliability of the process. The disclosed methods overcome calibration errors due to reagent instability and compensate for errors due to several instrument, assay environment and procedural variables.

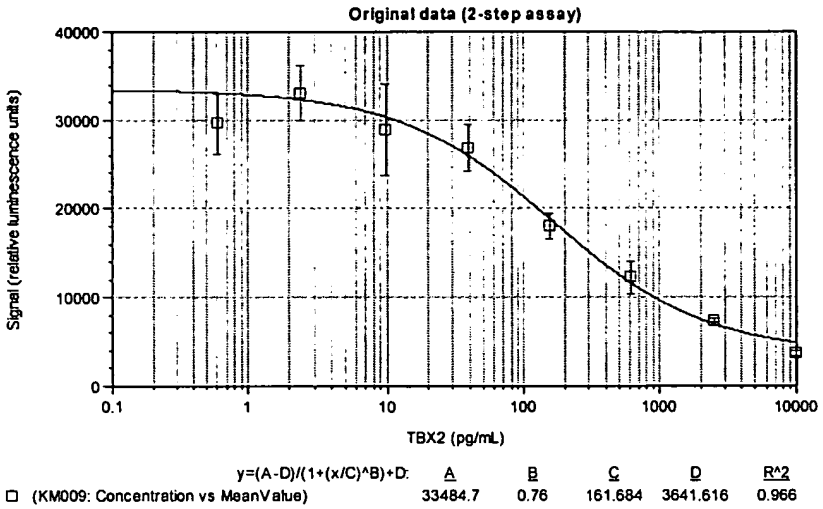

FIG. 1: Typical assay dose-response data for a two-step assay for TxB2

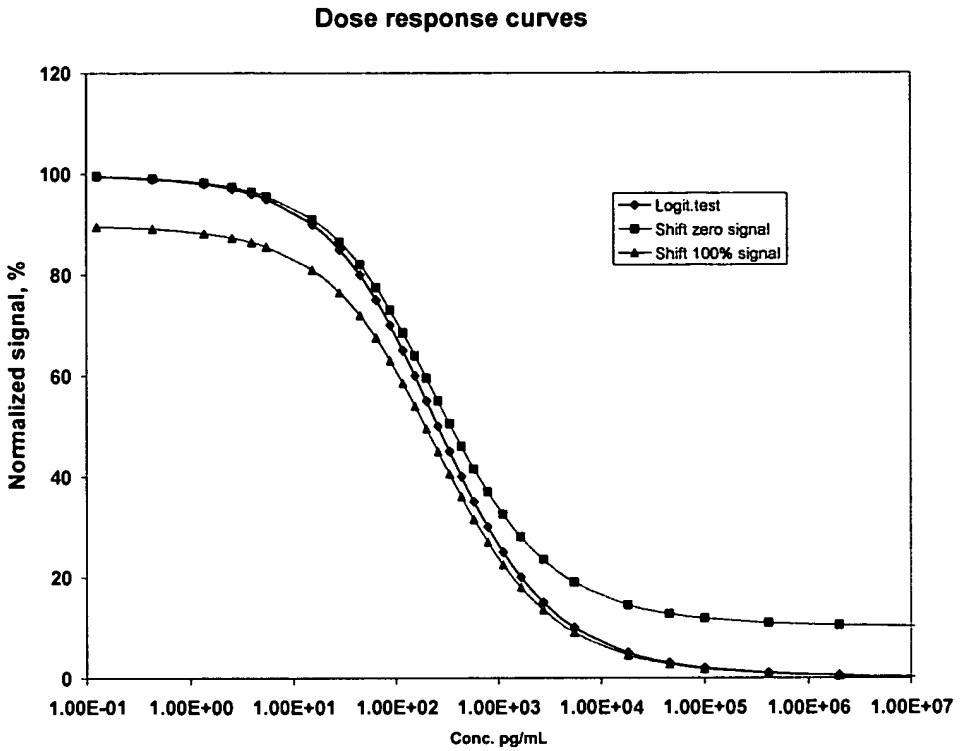

FIG. 2: Dose responses computed with and without errors in calibration parameters.

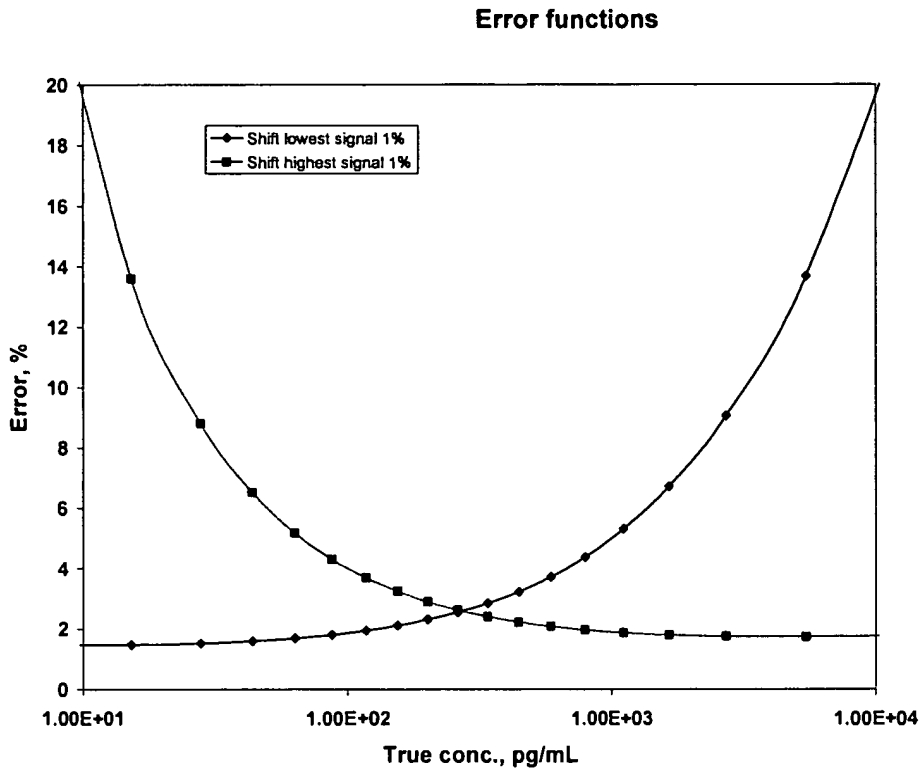

FIG. 3: Computed concentration errors produced by 1% mis-estimation of A and D calibration values

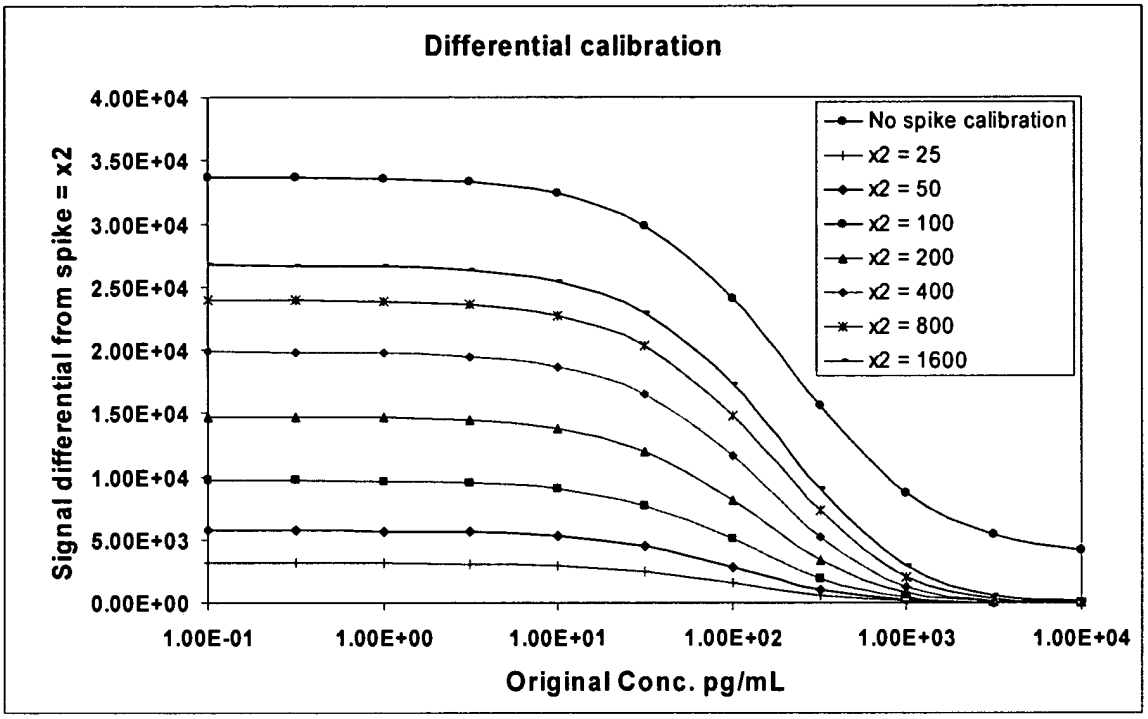

FIG. 4: Calibration using a "differential" approach

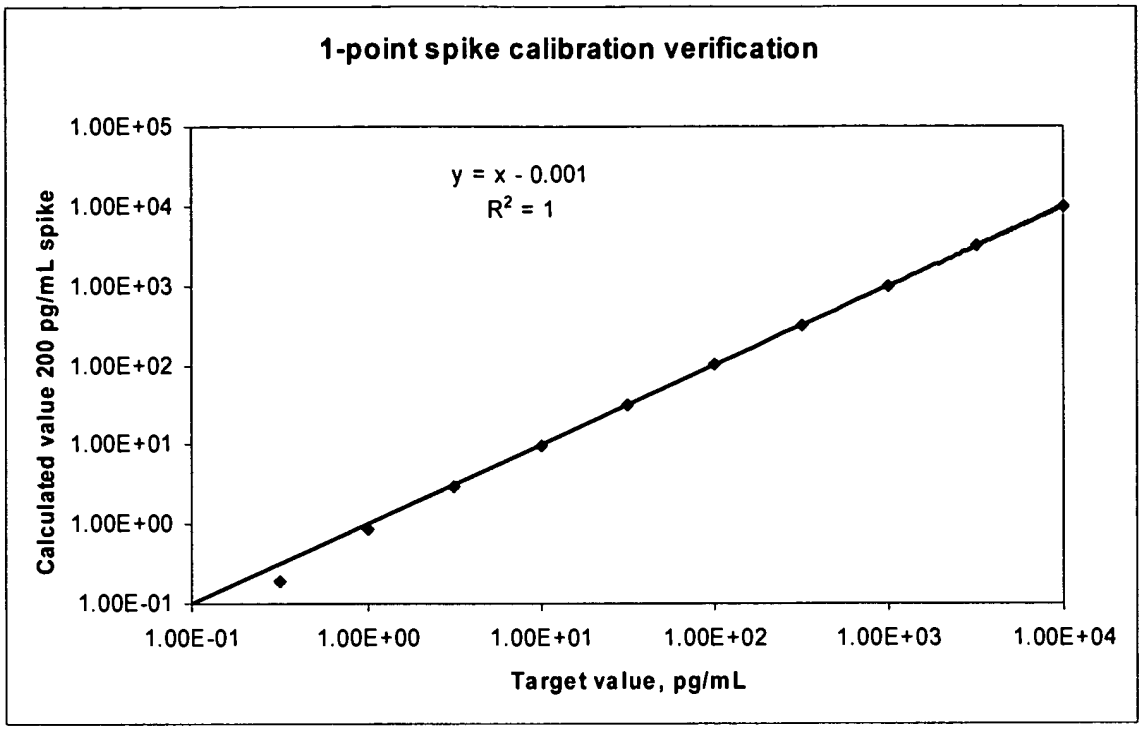

FIG. 5: Verification of calibration using the "1-point spike" method (log scale)

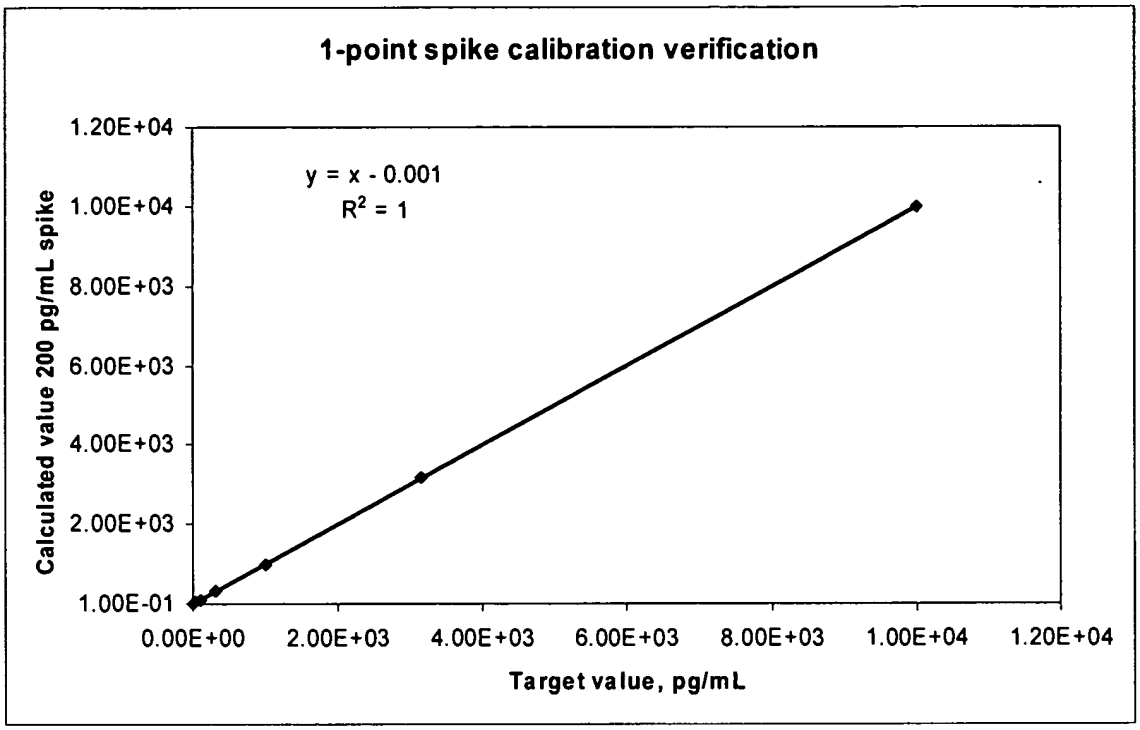

FIG. 6: Verification of calibration using the "1-point spike" method (linear scale)

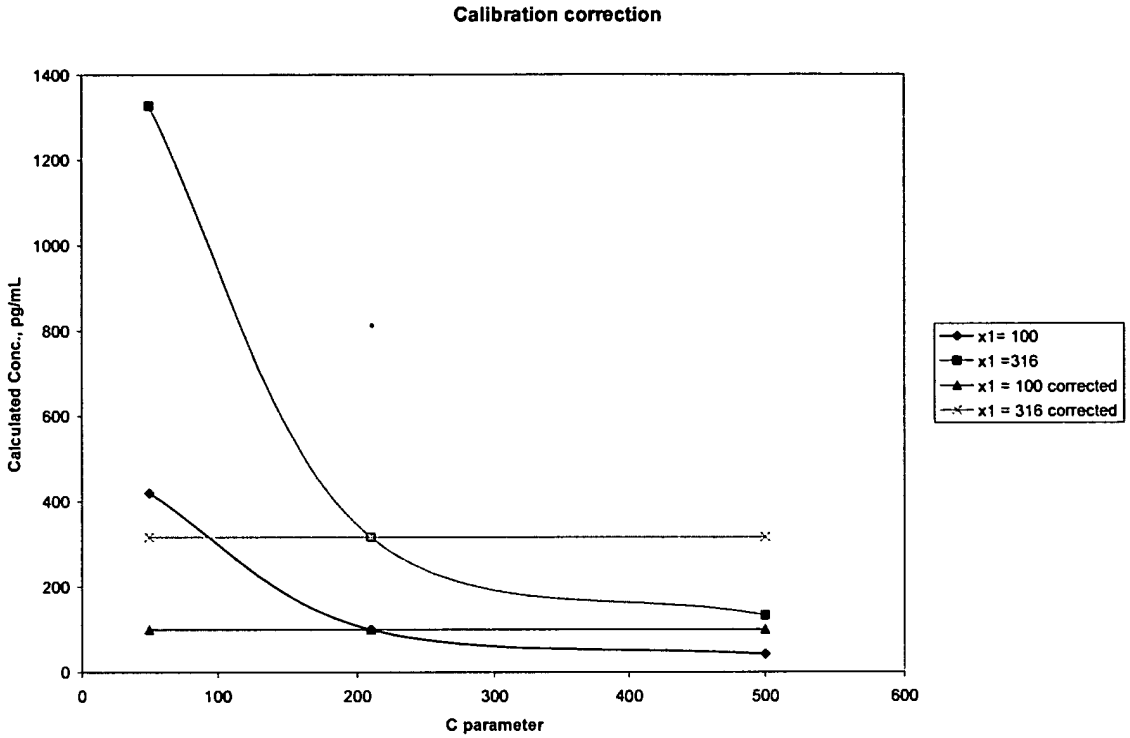

FIG. 6a: Using spike recovery to eliminate calibration errors of the "C" parameter.

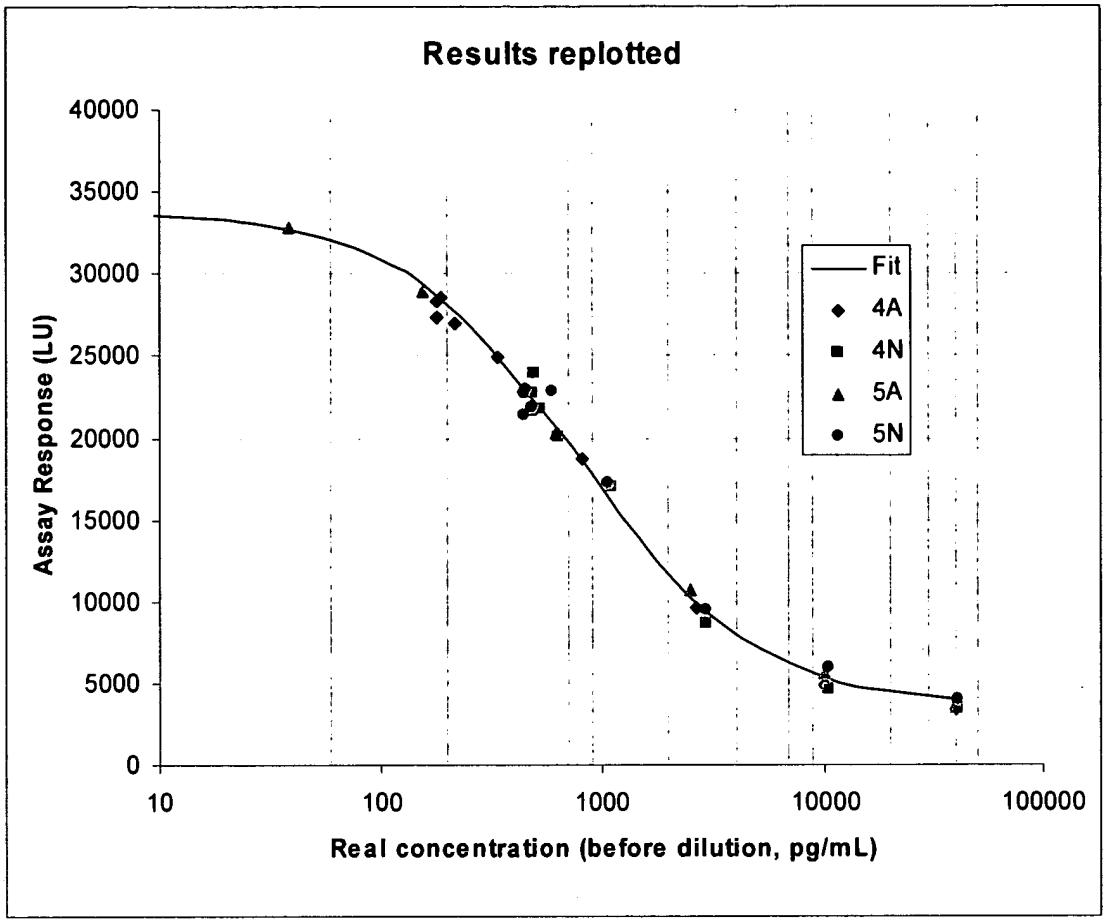

FIG. 7: Dose-response of assays calibrated against a plasma sample with a very low TxB2 concentration

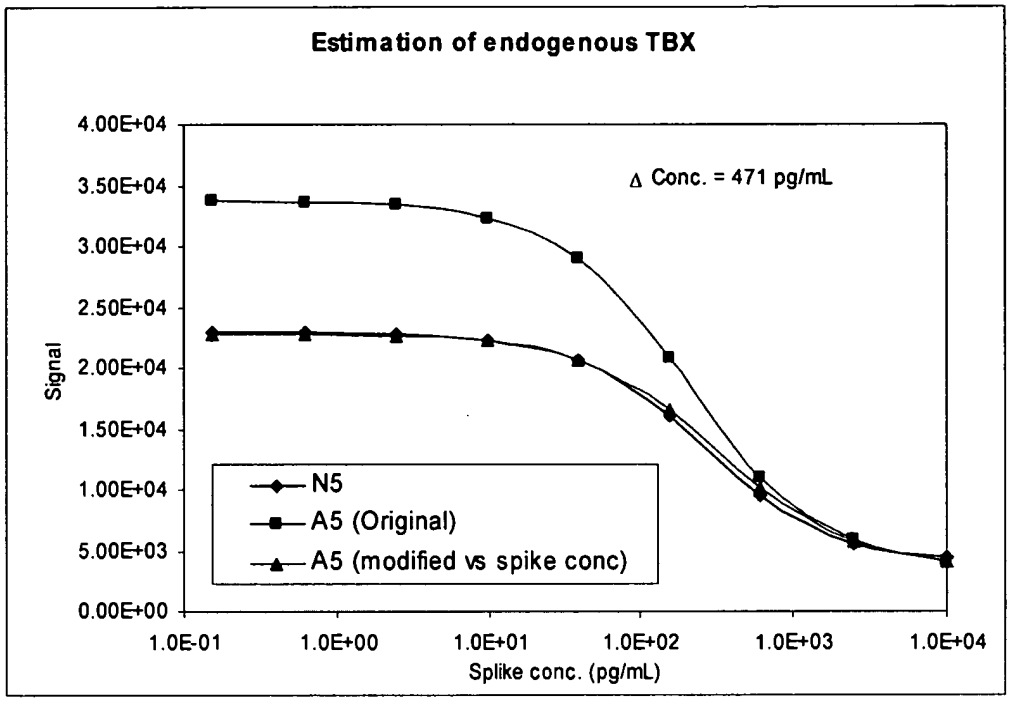

FIG. 8: Calculating difference in concentration between two samples

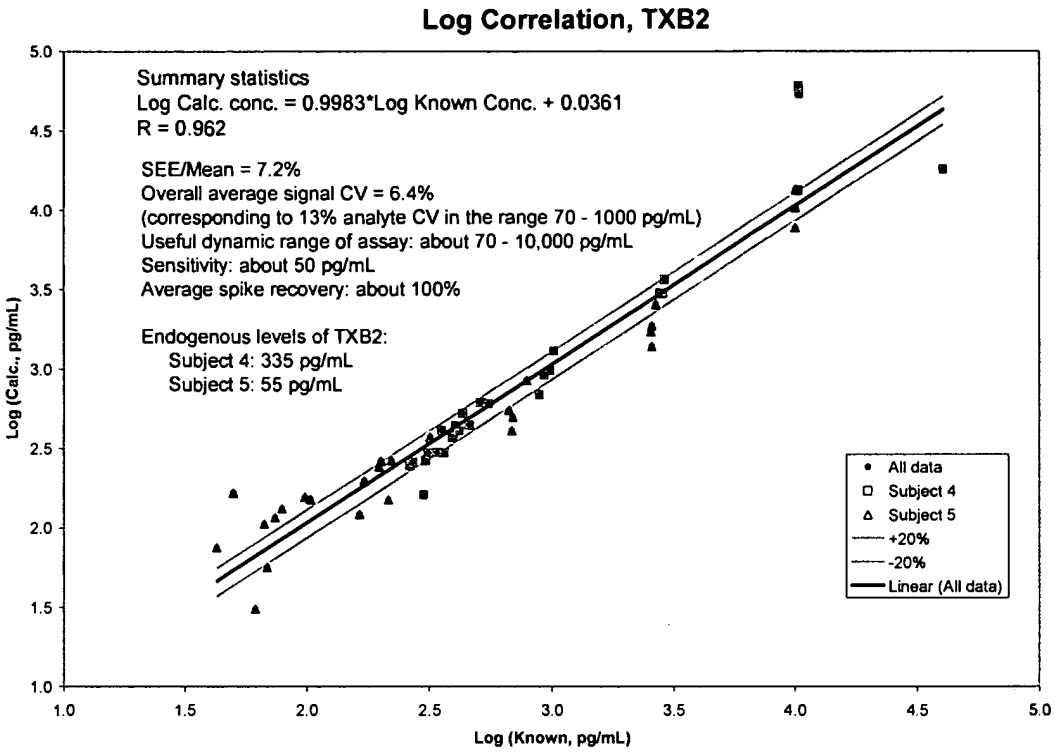

FIG. 9: Assay of plasma samples

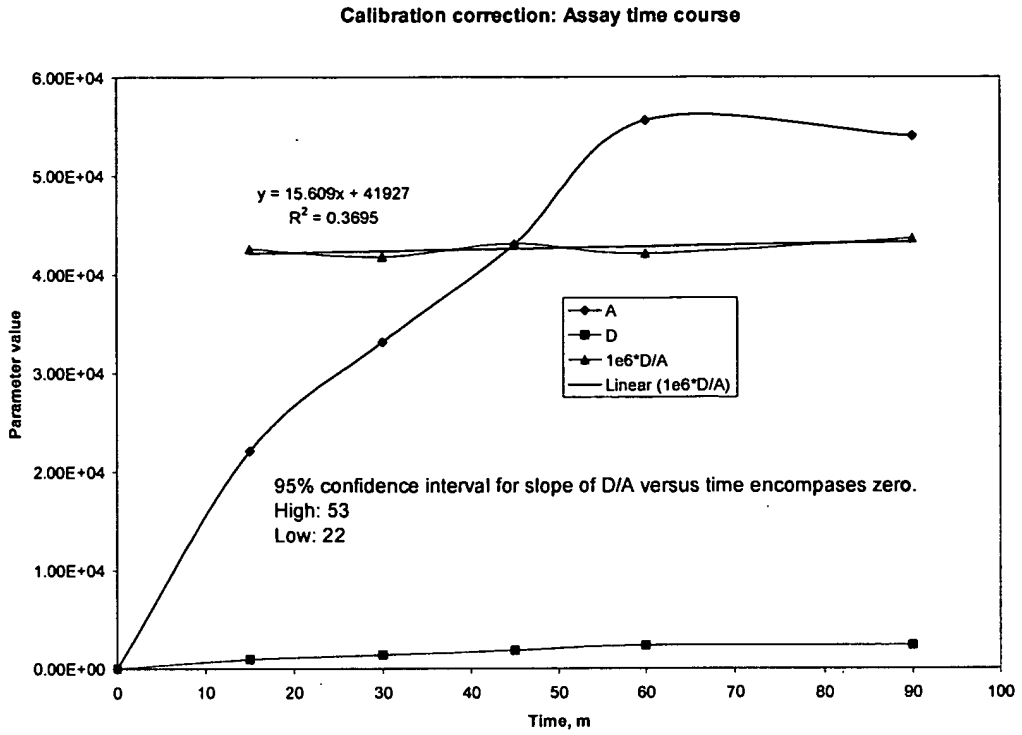

FIG. 10: Time course of assay signal generation

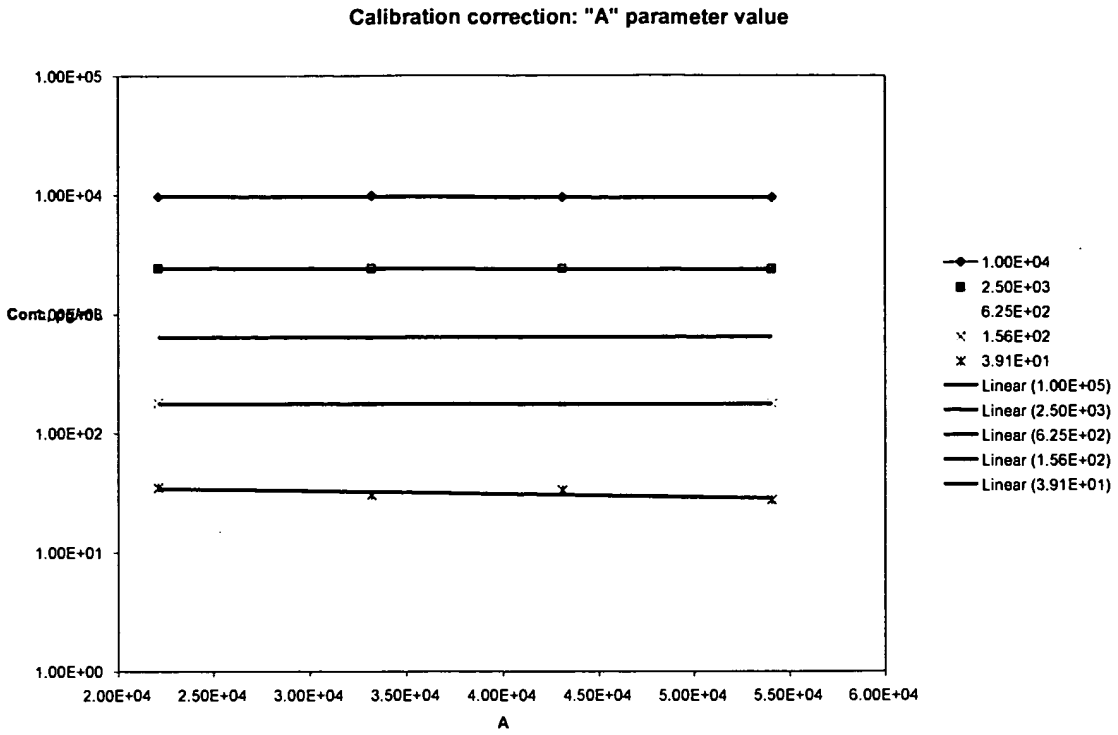

FIG. 11: Impact of change in calibration parameter A on assay calibration

PATENT APPLICATION SERIAL NO _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

08/09/2005 AADOF01 00000012 500417 60705489
01 FC:2005 100.00 DA

PTO-1556
(5/87)

*U.S. Government Printing Office: 2002 — 466-247/69033

EXHIBIT C

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
60/717,192	09/16/2005	Elizabeth Holmes	30696-705.101

CONFIRMATION NO. 7408

OC000000019088756

021971
WILSON SONSINI GOODRICH & ROSATI
650 PAGE MILL ROAD
PALO ALTO, CA 94304-1050

Date Mailed: 06/02/2006

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/12/2006.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

MELKAM BEYENE
PTOSS (703) 305-3006

OFFICE COPY

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
60/717,192	09/16/2005	Elizabeth Holmes	035738-0019

CONFIRMATION NO. 7408

OC000000019088748

20277
MCDERMOTT WILL & EMERY LLP
600 13TH STREET, N.W.
WASHINGTON, DC 20005-3096

Date Mailed: 06/02/2006

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/12/2006.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

MELKAM BEYENE
PTOSS (703) 305-3006

OFFICE COPY

ASSIGNMENT OF APPLICATION	Docket Number 30696-705.101

Whereas, the undersigned:

1. Holmes, Elizabeth A.
   Palo Alto, CA 94301
2. Roy, Shaunak
   San Mateo, CA 94403
3. Howard, John
   Saratoga, CA 95070
4. Wang, Chengwang
   Mountain View, CA 94043
5. Gibbons, Ian
   Portola Valley, CA 94028
6. Kemp, Tim
   San Jose, CA 95120

hereinafter termed "Inventors", have invented certain new and useful improvements in

SYSTEM AND METHOD FOR DETERMINING EFFICACY OF THERAPY AND MEDICATIONS IN INDIVIDUALS

☒ for which an application for United States Patent was filed on September 16, 2005 Application No. 60/717,192.
☐ for which a United States Patent issued on ___, U.S. Patent No. ___.

WHEREAS, Theranos, Inc., a corporation of the State of Delaware, having a place of business at 1430 O'Brien Drive, Suite H, Menlo Park, CA 94025, (hereinafter termed "Assignee"), is desirous of acquiring the entire right, title and interest in and to said application and the invention disclosed therein, and in and to all embodiments of the invention, heretofore conceived, made or discovered jointly or severally by said Inventors (all collectively hereinafter termed "said invention"), and in and to any and all patents, inventor's certificates and other forms of protection (hereinafter termed "patents") thereon granted in the United States and foreign countries.

NOW, THEREFORE, in consideration of good and valuable consideration acknowledged by said Inventors to have been received in full from said Assignee:

1. Said Inventors do hereby sell, assign, transfer and convey unto said Assignee the entire right, title and interest (a) in and to said application and said invention; (b) in and to all rights to apply for foreign patents on said invention pursuant to the International Convention for the Protection of Industrial Property or otherwise; (c) in and to any and all applications filed and any and all patents granted on said invention in the United States or any foreign country, including each and every application filed and each and every patent granted on any application which is a divisional, substitution, continuation, or continuation-in-part of any of said applications; and (d) in and to each and every reissue or extensions of any of said patents.

2. Said Inventors hereby jointly and severally covenant and agree to cooperate with said Assignee to enable said Assignee to enjoy to the fullest extent the right, title and interest herein conveyed in the United States and foreign countries. Such cooperation by said Inventors shall include prompt production of pertinent facts and documents, giving of testimony, execution of petitions, oaths, specifications, declarations or other papers, and other assistance all to the extent deemed necessary or desirable by said Assignee (a) for perfecting in said Assignee the right, title and interest herein conveyed; (b) for prosecuting any of said applications; (c) for filing and prosecuting substitute, divisional, continuing or additional applications covering said invention; (d) for filing and prosecuting applications for reissuance of any said patents; (e) for interference or other priority proceedings involving said invention; and (f) for legal proceedings involving said invention and any applications therefor and any patents granted thereon, including without limitation reissues and reexaminations, opposition proceedings, cancellation proceedings, priority contests, public use proceedings, infringement actions and court actions; provided, however, that the expense incurred by said Inventors in providing such cooperation shall be paid for by said Assignee.

3. The terms and covenants of this assignment shall inure to the benefit of said Assignee, its successors, assigns and other legal representatives, and shall be binding upon said Inventors, their respective heirs, legal representatives and assigns.

4. Said Inventors hereby jointly and severally warrant and represent that they have not entered and will not enter into any assignment, contract, or understanding in conflict herewith.

IN WITNESS WHEREOF, said Inventors have executed and delivered this instrument to said Assignee as of the dates written below:

Date: April 19, 2006
Elizabeth A. Holmes

Date: 04/28/06
Shaunak Roy

Date: 05/31/06
John Howard

Date: 4/25/06
Chengwang Wang

Date: 4/25/2006
Ian Gibbons

Date: 04/25/06
Tim Kemp

Practitioner's Docket No.: 30696-705.101 ***PATENT***

POWER OF ATTORNEY BY ASSIGNEE TO EXCLUSION OF INVENTOR UNDER 37 C.F.R. § 3.71 WITH REVOCATION OF PRIOR POWERS

The undersigned ASSIGNEE of the entire interest in:

☐ U.S. Patent No.
☒ U.S. application no. 60/717,192, filed on September 16, 2005

hereby appoints all Wilson Sonsini Goodrich & Rosati attorneys registered to practice before the United States Patent and Trademark Office, as associated with:

Customer No. 021971

to prosecute this application and transact all business in the United States Patent and Trademark Office in connection therewith and hereby revokes all prior powers of attorney; said appointment to be to the exclusion of the inventors and the inventors' attorneys in accordance with the provisions of 37 C.F.R. § 3.71.

The following evidentiary documents establish a chain of title from the original owner to the Assignee:

(complete one of the following)

☒ a copy of an Assignment attached hereto, which Assignment has been (or is herewith) forwarded to the Patent and Trademark Office for recording; or

☐ the Assignment recorded on ___ at reel ___, frames ___-___.

Pursuant to 37 C.F.R. § 3.73(b) the undersigned Assignee hereby states that evidentiary documents have been reviewed and hereby certifies that, to the best of ASSIGNEE's knowledge and belief, title is in the identified ASSIGNEE.

CHANGE OF CORRESPONDENCE ADDRESS

Direct all correspondence and telephone calls to:

Name	Karen K. Wong, Ph.D. J.D.					
Address	Wilson Sonsini Goodrich and Rosati					
Address	650 Page Mill Road					
City	Palo Alto	State	CA	Zip	94304	Customer No.: 021971
Country	USA	Telephone	(650) 493-9300	Fax	(650) 493-6811	

ASSIGNEE: Theranos, Inc.

Name: Elizabeth Holmes
Print

Signature

Title: President and CEO

Date: April 25, 2006.

Electronic Acknowledgement Receipt

EFS ID:	1046066
Application Number:	60717192
Confirmation Number:	7408
Title of Invention:	System and methods for determining efficacy of therapy and medications in individuals
First Named Inventor:	Elizabeth Holmes
Customer Number:	20277
Filer:	Vernon A. Norviel
Filer Authorized By:	
Attorney Docket Number:	035738-0019
Receipt Date:	12-MAY-2006
Filing Date:	16-SEP-2005
Time Stamp:	15:00:21
Application Type:	Provisional
International Application Number:	

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)	Multi Part	Pages
1	Power of Attorney (may include Associate POA)	30696-705-101POA.pdf	124443	no	2

Warnings:	
Information:	
Total Files Size (in bytes):	124443

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

Docket No.: 035738-0019 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Customer Number: 20277
Elizabeth Holmes, et al.	:	Confirmation Number: 7408
Application No.: 60/717,192	:	Group Art Unit: Not yet assigned
Filed: September 16, 2005	:	Examiner: Not yet assigned

For: SYSTEM AND METHODS FOR DETERMINING EFFICACY OF THERAPY AND MEDICATIONS IN INDIVIDUALS

REQUEST FOR CORRECTED FILING RECEIPT

Mail Stop OFR
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Attached is a copy of the Filing Receipt received from the U.S. Patent and Trademark Office in the above-referenced application. It is noted that there is an **error in the title**. Attached is a copy of the Provisional Patent Application Cover Sheet, which evidences the **correct title is "System and methods for determining efficacy of therapy and medications in individuals**". It is requested that a corrected filing receipt be issued.

Respectfully submitted,

McDERMOTT WILL & EMERY LLP

Judith L. Toffenetti
Registration No. 39,048

600 13th Street, N.W.
Washington, DC 20005-3096
Phone: 202.756.8000 JLT:sln
Facsimile: 202.756.8087
Date: October 12, 2005

Please recognize our Customer No. 20277 as our correspondence address.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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APPL NO.	FILING OR 371 (c) DATE	ART UNIT	FIL FEE REC'D	ATTY.DOCKET NO	DRAWINGS	TOT CLMS	IND CLMS
60/717,192	09/16/2005		100	035738-0019	7		

CONFIRMATION NO. 7408

20277
MCDERMOTT WILL & EMERY LLP
600 13TH STREET, N.W.
WASHINGTON, DC 20005-3096

FILING RECEIPT

OC000000017107931

Date Mailed: 09/27/2005

Receipt is acknowledged of this provisional Patent Application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please mail to the Commissioner for Patents P.O. Box 1450 Alexandria Va 22313-1450. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Elizabeth Holmes, Palo Alto, CA;
Shaunak Toy, San Mateo, CA;
John Howard, Saratoga, CA;
Chengwang Wang, Mountain View, CA;
Ian Gibbons, Portola Valley, CA;
Tim Kemp, San Jose, CA;

Power of Attorney: The patent practitioners associated with Customer Number **20277**.

If Required, Foreign Filing License Granted: 09/26/2005

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is US60/717,192

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

System and method for determining efficacy of therapy and medications in individuals

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER
Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

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The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

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national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

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No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Express Mail Label No.			Docket Number	035738-0019
INVENTOR(s)/APPLICANT				
LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (City and Either State or Foreign Country)	
HOLMES	Elizabeth		Palo Alto, CA	
TOY	Shaunak		San Mateo, CA	
HOWARD	John		Saratoga, CA	
WANG	Chengwang		Mountain View, CA	
GIBBONS	Ian		Portola Valley, CA	
KEMP	Tim		San Jose, CA	
Additional inventors are being named on the separately numbered sheets attached hereto.				
TITLE OF THE INVENTION (500 characters max)				
SYSTEM AND METHODS FOR DETERMINING EFFICACY OF THERAPY AND MEDICATIONS IN INDIVIDUALS				
CORRESPONDENCE ADDRESS				
McDERMOTT WILL & EMERY LLP 600 13th Street, N.W. Washington, D. C. 20005-3096 202.756.8000				
STATE	Washington, D. C.	**ZIP CODE**	20005-3096	**COUNTRY** USA

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of pages* [20] ☒ Small Entity Statement

☒ Drawings *Number of sheets* [7] ☐ Other (specify): _____

Application Size Fee: If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CAR 1.16(s).

METHOD OF PAYMENT OF APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27. TOTAL FEE ($)

☐ A check or money order is enclosed to cover the filing fee and application size fee (if applicable). $100.00

☒ The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number: 500417 .

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

McDERMOTT WILL & EMERY LLP

Judith L. Toffenetti
Registration No. 39,048

600 13th Street, N.W.
Washington, DC 20005-3096
Phone: 202.756.8000 JLT:aph
Facsimile: 202.756.8087

Date: September 16, 2005

Please recognize our Customer No. 20277 as our correspondence address.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Express Mail Label No.			Docket Number	035738-0019

INVENTOR(s)/APPLICANT

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (City and Either State or Foreign Country)
HOLMES	Elizabeth		Palo Alto, CA
TOY	Shaunak		San Mateo, CA
HOWARD	John		Saratoga, CA
WANG	Chengwang		Mountain View, CA
GIBBONS	Ian		Portola Valley, CA
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TITLE OF THE INVENTION (500 characters max)

SYSTEM AND METHODS FOR DETERMINING EFFICACY OF THERAPY AND MEDICATIONS IN INDIVIDUALS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of pages* [20] ☒ Small Entity Statement
☒ Drawings *Number of sheets* [7] ☐ Other (specify): _____

Application Size Fee: If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CAR 1.16(s).

METHOD OF PAYMENT OF APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.

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Respectfully submitted,

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SYSTEM AND METHODS FOR DETERMINING EFFICACY OF THERAPY AND MEDICATIONS IN INDIVIDUALS

Field of the Invention

[0001] This invention relates generally to real-time diagnostic and therapeutic monitoring. More particularly, it relates to predicting and preventing adverse reactions to drugs and determining the efficacy of drug therapies.

Description of the Related Art

[0002] Adverse Drug Reactions (ADRs) are one of the leading causes of morbidity and mortality in health care. The Institute of Medicine reported in January 2000 that 44,000 to 98,000 deaths occur annually due to medical errors. Of this total, an estimated 7,000 deaths occur due to ADRs. To put this in perspective, 6,000 Americans die each year from workplace injuries. Other studies conducted on hospitalized patient populations have placed much higher estimates on the overall incidence of serious ADRs. These studies estimate that 6.7% of hospitalized patients have a serious adverse drug reaction with a fatality rate of 0.32%. If these estimates are correct, then there are more than 2,216,000 serious ADRs in hospitalized patients, causing over 106,000 deaths annually. If true, then ADRs are the 4th leading cause of death in the U.S.—ahead of pulmonary disease, diabetes, AIDS, pneumonia, accidents, and automobile deaths. These statistics do not include the number of ADRs that occur in ambulatory settings. Also, it is estimated that over 350,000 ADRs occur in U.S. nursing homes each year. The exact number of ADRs is not certain and is limited by methodological considerations. However, whatever the true number is, ADRs represent a

significant public health problem that is, for the most part, preventable. Additionally, health care costs associated with adverse drug reactions are astronomical. While there are limitations to accurately estimating such costs, one estimate of the cost of drug-related morbidity and mortality is $136 billion annually (**Johnson JA**, Bootman JL. Drug-related morbidity and mortality. A cost-of-illness model. *Arch Intern Med* 1995;155(18):1949–1956), which is more than the total cost of cardiovascular or diabetic care in the United States. In addition, one out of 5 injuries or deaths per year of hospitalized patients may be due to ADRs. Finally, a two-fold greater mean length of stay, cost and mortality has been reported for hospitalized patients experiencing an ADR compared to a control group of patients without an adverse drug reaction (**Classen DC** , Pestotnik SL, Evans RS, Lloyd JF, Burke JP. Adverse drug events in hospitalized patients. Excess length of stay, extra costs, and attributable mortality. *JAMA* 1997;277(4):301–306).

[0003] A business aspect of ADRs is the adverse impact ADRs have on marketed products. Recently, we have experienced the dramatic impact ADRs can have on the viability of billion dollar drugs. After it had been on the market for many years and had generated many billions of dollars in revenues, Merck had to pull Vioxx (a COX2 inhibitor) off the market, because of ADRs.

[0004] There are many reasons for the prevalence of ADRs. Here are just a few. First, more drugs—and many more combinations of drugs—are being used to treat patients than ever before. To exemplify this point, 64% of all patient visits to physicians result in prescriptions (**Schappert SM**. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States,1997. National Center for Health Statistics. *Vital Health Stat.* 1999;13(143). Second, 2.8 billion prescriptions were filled in the year 2000 (**National Association of Chain Drug Stores**. 2000 community pharmacy results. 2001, Alexandria, VA). That is about 10 prescriptions for every person in the United States.

Finally, the rate of ADRs increases exponentially after a patient is on 4 or more medications (**Jacubeit T**, Drisch D, Weber E. Risk factors as reflected by an intensive drug monitoring system. *Agents Actions* 1990;29:117–125). Furthermore, efforts to reduce polypharmacy (simultaneous intake of many drugs) are important but for many patients, the number of medications cannot always be reduced without doing harm.

[0005] There is also an increasing trend towards the chronic use of drugs (statins such as Lipitor and Cox-2 inhibitors such as Vioxx and the like). The risks due to a drug may increase over a long period in an unpredictable patient-specific fashion. Chronic use of drugs also increases the chance that various changes in the patient's lifestyle, health status and use of other medications will occur. In women, the chronic use of drugs can result in unanticipated consequences, if the woman becomes pregnant. Such risks are of particular concern regarding the fetus, which is especially susceptible to ADRs including teratogenicity . A system that can identify such undesirable consequences in women can be especially beneficial for women who may become or more especially wish to become or are pregnant, enabling them to use drugs that could not otherwise be considered as usable in women who may be pregnant or wish to become pregnant]

[0006] Estimates of the numbers of patients injured due to drug interactions vary widely. In a systems analysis of ADRs, one publication estimated that drug-drug interactions represent from 3–5% of all in-hospital medication errors (**Leape LL,** Bates DW, Cullen DJ, Cooper J, Demonaco HJ, Gallivan T, et al. Systems analysis of adverse drug events. ADE Prevention Study Group. *JAMA* 1995;274(1):35–43). Drug interactions are also an important cause of patient visits to emergency departments (**Raschetti R,** Morgutti M, Menniti-Ippolito F, Belisari A, Rossignoli A, Longhini P, et al. Suspected adverse drug events requiring emergency department visits or hospital admissions. *Eur J Clin Pharmacol* 1999;54(12):959–963).

[0007] A further important factor in managing the risks and benefits of drug therapy is patient compliance. Patients (a) often fail to take scheduled dose of drug, (b) frequently take an inappropriate dose, (c) take more than the prescribed number of pills in a day or (d) fail to complete a course of drug therapy (especially common in treatment for infectious disease). These behaviors (deliberate or inadvertent) result in improper levels of drugs in the body with likely adverse effects. The patient is typically oblivious to such consequences and the prescribing physician is also unlikely to realize the problem. A system that is capable of identifying such problems could be very useful.

[0008] Recent publications have shown that many adverse drug reactions can be prevented and detected through the use of systems interventions. For example, many health systems have instituted new technologies to minimize patient injury due to medication errors and drug-drug interactions (**Bates DW**, Leape LL, Cullen DJ ,Laird N, Petersen LA, Teich JM et al. Effect of computerized physician order entry and a team intervention on prevention of serious medication errors. *JAMA* 1998;280(15):1311–1316; **Evans RS**, Pestotnik SL, Classen DC, Horn SD, Bass SB, Burke JP. Preventing adverse drug events in hospitalized patients. *Ann Pharmacother* 1994;28(4):523–527; **Gebhart F**. VA facility slashes drug errors via bar-coding. *Drug Topics* 1999;1:44). Tools like computerized physician order and prescription entry and bar coding systems have demonstrated tangible benefits. The potential for reducing medication errors by using computerized medical records as well as drug-interaction screening software that detects and alerts the physician and/or pharmacist to potentially serious drug interactions has been recognized.

[0009] These technological solutions do have limitations, however. The fragmentation of healthcare delivery may result in incomplete records. More significant is the fact that, although this information is available, it is not uniformly or optimally incorporated into decision making. This is exemplified in the observation by Cavuto et al. that pharmacists

filled prescriptions for drug combinations that were known to interact even though computerized drug interaction software was in place (**Committee on Quality of Health Care in America**: Institute of Medicine. To err is human: building a safer health system. Washington, D.C.: National Academy Press,2000. (**Cavuto NJ**, Woosley RL, Sale M. Pharmacies and prevention of potentially fatal drug interactions. *JAMA* 1996;275: 1086–1087. This problem persists as shown in the 2000 paper by Smalley et al. on prescription errors with cisapride (**Smalley W**, Shatin D, Wysowski DK, Gurwitz J Andrade SE, Goodman M, et al. Contraindicated use of cisapride: impact of food and drug administration regulatory action. *JAMA* 2000;284(23):3036–3039).

[0010] Some of the current limitations in preventing ADRs are the inability to monitor drugs, their metabolites and relevant biomarkers in ambulatory patients, and particularly from whole blood and on frequent test basis.

[0011] Even those approaches that describe personalized medicine, rely on monitoring genetic variation or basal-level activity of targeted markers. What is needed is a system that does not differentiate on a population basis. Instead, a system that treats each individual separately, enabling therapeutic customization *after* each patient has been dosed with a drug.

[0012] Thus, there is a need for a system for determining in real-time the individual concentrations of drugs, their metabolites and biological markers that are related to those drugs and their analytes and methods for correlating the measured parameters with outcomes and providing the healthcare provider an early warning system based on observed trends in the measured parameters. Additionally, it is desirable to profile targeted analytes and biomarkers in small blood samples of patients. Such profiling could assist in determining ADRs and undesirable drug-drug interactions.

[0013] The current spending on medicines in this country exceeds $300 billion. However, generally, there are not many objective measurements that are used to measure the

efficacy of these drug treatments. Usually, clinical endpoints, such as lowered blood pressure in the case of medications for treating high blood pressure, or lowered cholesterol levels (in the case of medications for treating high cholesterol levels) are used to determine the efficacy of the medications. However, it is well known that the clinical endpoint could be influenced by various parameters, including the patient's diet. It is possible that the medication is having the desired impact at the molecular level, but the outcome at the physiological level is negatively influenced by non-therapeutic activities, such as diet. In such a situation, it will be useful to know that the medication is having its desired effect and that the other parameters, whether it is diet or other drugs, are negatively influencing the outcome of the drug treatment.

[0014] Generally, a therapeutic intervention could be (a) effective, (b) ineffective, or (c) have serious side effects. Beyond determining ADRs, it will be very useful to objectively determine the efficacy of treatments based on markers that are influenced by the therapy. Such markers could be concentrations of the drugs, their metabolites, or biological markers such as proteins that are expressed due to the treatment. Furthermore, patient's other activities, such as diet, exercise, smoking, etc., could influence the efficacy of the treatment. It will be beneficial to the patient, the healthcare provider and the drug manufacturers to be able to identify the interactions of all the various parameters that influence the outcome of the therapy, by monitoring the various parameters in real-time and computing the interactions between those monitored parameters.

Summary of the Invention

[0015] The present invention is for determining efficacy of medical treatments and overcomes limitations of existing methods in predicting and preventing adverse drug interactions. The method comprises computing a therapeutic index (TI) by (1) measuring a

defined combination of parameters such as the concentration in blood (and/or blood plasma) of one or more (a) drugs that are consumed by a patient and their metabolites, (b) other analytes (cell numbers cell surface markers, etc., or (c) biomarkers and (2) calculating the TI using an equation derived from preliminary or concurrent studies preformed in a large set of patients undergoing similar treatments and/or normal (control) subjects and involving measurement of the analytical parameters and one or more physiological parameters (such as blood pressure) or "outcome parameters" such as an adverse cardiac event and comparing the computed therapeutic index with a stored "action threshold value".

[0016] A system for computing a therapeutic index of a therapy in an ambulatory patient by measuring in a portable instrtument the concentration in blood of one or more (a) drugs that are consumed by a patient and their metabolites, (b) analytes, or (c) biomarkers and comparing the computed therapeutic index with a action threshold value stored in a database, wherein the therapeutic index function has been derived from a historic analysis of various parameters from patients undergoing similar treatments and is compared with a stored action threshold value that has been determined by analysis of various parameters from patients undergoing similar treatments.

[0017] In one embodiment, the invention comprises measuring one or more markers from a patient's blood (device input) in an ambulatory setting, communicating that measurement to a server; the patient or the patient's healthcare provider (patient input) communicating to the server, on a periodic basis, personal information relating to medications and foods the patient is consuming, and computing a therapeutic index at the server. Additionally, the computed therapeutic index could be compared to a reference therapeutic index to determine whether the treatment that the patient is undergoing is effective or ineffective.

[0018] Other aspects of the invention include methods corresponding to the devices and systems described above.

Brief Description of the Drawings

[0019] The invention has other advantages and features which will be more readily apparent from the following detailed description of the invention and the appended claims, when taken in conjunction with the accompanying drawings, in which:

[0020] FIGs. 1-3 illustrate how the therapeutic index would be computed.

[0021] FIGs. 4-5 illustrate the relationship between measured concentrations of drug, analyte and biomarker and therapeutic index and application of this invention to minimize ADRs.

[0022] FIG. 6 shows a screen shot of how a patient could provide the input parameters.

[0023] FIG. 7 illustrates the use of TI to follow treatment progression in an autism patient.

Detailed description of the Preferred Embodiments

[0024] Although the detailed description contains many specifics, these should not be construed as limiting the scope of the invention but merely as illustrating different examples and aspects of the invention. It should be appreciated that the scope of the invention includes other embodiments not discussed in detail above. Various other modifications, changes and variations which will be apparent to those skilled in the art may be made in the arrangement, operation and details of the method and apparatus of the present invention disclosed herein without departing from the spirit and scope of the invention as described here.

[0025] The concept of the reference therapeutic index and how it is computed is illustrated in FIGs. 1 and 2. A therapeutic index (TI) is computed from a retrospective

analysis of many measured parameters, including the blood concentrations of drugs of interest, their metabolites, other analytes and biomarkers in blood that change concentrations due to the drugs the patient is consuming, physiologic parameters (such as blood pressure, respiratory rate, body temperature, heart rate, etc.), and clinical parameters that indicate disease progression (such as angina, stroke, infarct, etc.). Typically, many serial measurements would be made for the many treated patient and corresponding controls (unmedicated or placebo treated). The clinical parameter would be an "outcome parameter" (OP). The other measured parameters could be termed as "input parameters" (IP).

[0026] For the retrospective analysis and TI computation, data from many subjects and their respective output and input parameters, including subject's relevant details such as height, weight, race, sex, family history, etc., would be populated in a database. Each candidate outcome parameter (stroke, infarct, angina, death, etc.) will be subject to multiple regression analysis against input parameters.

[0027] The multiple regression analysis is performed for each candidate OP versus all available IPs. Database columns are constructed by using each IP, each IP^2, and all cross-terms (IPi*IPj).

[0028] The analysis is then performed using the equation:

OPi = (a*IP1+ b* IP2 + ... n*IPn) + (aa*IP1^2+bb*IP2^2+...+nn*IPn^2) + (aaa*IP1*IP2+bbb*IP1*IP3+...+nnn*IPn-1*IPn)

Where a...n, aa...nn, aaa...nnn are arbitrary constants.

[0029] Multiple regression analysis establishes the best fit to the equation and indicates which IPs are strong candidates for inclusion.

[0030] Weakly correlated IPs are dropped and the analysis repeated until each candidate OP has an optimal relation to the remaining IPs.

[0031] The therapeutic index will then have the form:

TI = a*IP+cc*IP3^2+nnn*IP3*IP5+... (Equation 1)

[0032] FIG.2 illustrates the computation of a TI and the use of the TI concept for determining therapeutic efficacy (the therapeutic index is also indicated by the term efficacy index). The test case illustrated in FIG. 2 shows the time course of successful drug therapy of a disease state (such as atherosclerosis) that is indicated by three biochemical analytes represented by parameters A, B and C. The disease is treated (with for example a Statin) starting on day zero.

[0033] Parameters A, B and C are measured daily using an ambulatory system as described in co-pending application nos. 60/678, 801, 60/705,489 and 11/202,206. At the outset, relative to "ideal levels", Parameter A (for example LDL-cholesterol) is elevated, Parameter B (for example HDL-cholesterol) is low and Parameter C (for example, alanine aminotransferase, an indicator of liver damage) is normal. All parameters (A, B, C) are presented normalized to their respective ideal level.

[0034] As therapy proceeds, the drug causes the levels of A and B to approach normal values but at different rates. Analyte C remains normal indicating the drug is not causing liver damage.

[0035] The relative risk of an outcome for the patient is represented by an initially unknown TI. As described above, TI is a surrogate to the outcome parameter that reflects the physiological functions of the patient (blood pressure, etc.) or other pre-identified factors in a patient record and can be indicative of improvement in the patient's condition. We further

assume that parameter TI is influenced by parameters A and B. In certain cases, at the beginning of the study this relationship remains to be determined.

[0036] Data from the monitoring system (device input) and the patient input are analyzed by multiple regression of TI and measured values A, B and C, as described above. In the example shown, these data are analyzed using multiple regression analysis, which fits parameter TI as a function of parameters A, B, C and their squares and the pair-wise cross terms (A*B, etc.) As shown in FIG. 3, for the simulated values shown in FIG. 2, an excellent fit was obtained (R^2 = 0.99) when all parameters were included. It is evident from inspection of the fit that most of the parameters can be eliminated leaving only A and A*B. When this is done the fit is still very good (R^2 = 0.95).

[0037] Note that the multiple regression derived function is not identical to the base function which generated the first candidate TI data, but works well to compute an estimate of TI from (typically fewer) measured parameters, prior to clinical validation, if necessary.

[0001] The appropriate threshold levels of TI, or the optimum TI is termed as TI_{ref} (or "action threshold value".) Expert review would then determine the optimum therapeutic index for that particular patient or patient class. If the computed TI exceeds the preset TI_{ref}, appropriate action can be taken. An appropriate action could be alerting the physician, stopping the medication or the like. As can be understood, the appropriate TI_{ref} for a patient would be decided based on the healthcare provider's judgment for that individual patient. The *form* of the TI is derived as a one time exercise using expert analysis of the data set derived from clinical studies and/or existing clinical information.

[0038] Once the TI_{ref} is identified, then the use of this parameter is illustrated in FIG. 4. Methods of measuring drug, analyte and biomarker concentrations and conducting a two-way communication with a database using a handheld device are described in detail in co-pending applications 60/678,801, 60/705,489 and 11/202,206. The time course of various measured

and computed parameters are shown in FIG. 4. The curve marked CBX Dose illustrates the time course of a drug that is taken on a regular basis. The plotted values are normalized to what would be considered as "ideal levels" for that measurement. For example, if the expected ideal blood concentration of CBX is 100 ng/ml and if the measured concentration in blood is 100 ng/ml, the parameter value is 1.0 (with no offset) for CBX. Similarly, the concentrations of CXB, a metabolite of CBX, biomarkers Tx-M and PGI-M, which vary in response to the concentrations of the drug and the disease state, are also normalized to their ideal values and plotted. All the drug, analyte and biomarker concentrations could be measured using a system as described in currently pending applications 60/678, 801, 60/705,489 and 11/202,206. As explained above, the TI_{ref} for this particular patient is plotted on FIG. 4 as a flat line. Using the parameter values (a....n, aa.....nn, aaa...nnn) of Equation 1 and the measured input parameters (IP), the current TI for the patient is calculated. If the computed TI exceeds the TI_{ref} value, then an alert is generated. The alert could be targeted to the patient's healthcare provider, who in turn can take the appropriate action. An appropriate action could be to watch the patient closely for other clinical indications and/or alter the dosage and drugs the patient is taking.

[0039] FIGs. 4 and 5 illustrate the concept as to how when the computed TI exceeds the TI_{ref} a proactive action could avert an ADR. In FIG. 5, the patient's TI exceeded TI_{ref} about day 15. The patient is monitored closely and as the TI values continue to increase after day 30, the physician intervenes and reduces the dosage. This action starts lowering the TI for the patient and ultimately retreats to an acceptable level about day 60.

[0040] As can be understood, one or more individuals or entities that are involved in the care of the patient (nurses, physicians, pharmacist, etc.) could be alerted when the computed TI exceeds the TI_{ref} so that they could take the appropriate action. Additionally,

trends could be discerned and appropriate action could be taken even before a TI reaches a particular value.

[0041] It should be noted that many different analytes and biomarkers could be measured and construed as input parameters, IPs, while computing the TI. Such a list is shown in Table 1. Additionally, the list can be expanded or modified depending on the disease area as well. The appropriate list of parameters relating to certain diseases and drug treatments, e.g., cancer and infectious diseases and patient on NSAIDS, are illustrated in Tables 2 - 4.

[0042] **Table 1: List of Biomarkers and Analytes for Computing the Therapeutic Index**

[0043]

Lipids
Total cholesterol
HDL-cholesterol
LDL-cholesterol
Triglycerides
Carbohydrates
Fasting glucose
Hemoglobin A1c
Acute phase marker
C-reactive protein
Myocardial infarct/angina/ischemia
Lactate
Troponin-i
CKMB
Nutritional status
Prealbumin
Albumin
Coagulation
β-Thromboglobulin
Platelet factor 4
Von Willebrand factor
PT
APTT
Other
Potassium
BUN

Alanine aminotransferase
Eicosanoids
2,3 Dinor-6-keto-PGF1α
2,3-Dinor-11-dehydro-TxB-1α
2,3-Dinor-5,6-dihydro-8-iso-PGF-2α
11-Dehydro-TxB-1a
8-Iso-PGF-2α
6-Keto-PGF-1α
Thromboxane-B2
Blood cells
Hematocrit
Total white cells
T-cells
B-cells
Drug related
Aspirin metabolite
Ibuprofen
Acetaminophen
Celicoxib
Physiologic
Blood pressure (systolic)
Blood pressure (diastolic)
Weight
Temperature
Coronary Disease Risk Prediction Score
Other
Exercise
Smoking
Diet

[0044] Table 2: List of Biomarkers and Analytes as Indicators for Different Cancers

Biomarkers	Cancer
YKL -40	Ovarian
PSA	Prostate
Shed membrane fragment-associated markers for endometrial and ovarian cancers.	
CA 15-3 (or CA 27.29)	Breast
CEA	
AFP, CA125, CA15-3, CA19-9	HRP-ELISA (absorbance read)
HCG-beta (beta chain)	Choriocarcinoma
Microglobulin -beta 2 subunit (b2-M)	Multiple Myeloma
The neural cell adhesion molecule (NCAM)	Small-Cell Lung cancer

[0045] Table 3: List of Biomarkers and Analytes as Indicators for Infectious Diseases

Anti-infectives

Drug levels
IgM antibodies
IgG antibodies
anti-LPS antibodies
CRP

Microbial antigens
- shed
- not shed
- pyrogens

Antibodies to bacteria, virus

Drug resistance
- Penicillinase
- Methcillin resistance

Bacterial toxins
- Staph toxins

Cytokines
Immunosuppression
- Cyclosporin
- anti-HIV antibody detection

[0046] Table 4: List of Biomarkers and Analytes as Indicators for Determining ADRs due to NSAIDs

NSAID Relative
Thromboxane Relative
Prostocycline Relative
Total Cholesterol
HDL-Cholesterol
LDL-Cholesterol
Triglycerides
Fasting Glucose
Hemoglobin A1c
C-reactive protein
Lactate
Creatine kinase MB

Troponin-i
Prealbumin
Albumin
Beta-Thromboglobulin
Platelet Factor 4
Von Willebrand factor
Prothrombin Time
Activated Partial Thromboplastin Time
Potassium
Blood Urea Nitrogen
Alanine aminotransferase
2,3-Dinor-6-keto-PGF-1-alpha
2,3-Dinor-11-dehydro-TxB-1 alpha
2,3-Dinor-5,6-dihydro-8-ios-PGF-2 alpha
11-Dehydro-TxB-1a
8-Iso-PGF-2 alpha
Hematocrit
Total white cells
T-cells
B-cells
Aspirin Metabolite
Ibuprofen
Acetaminophen
Blood pressure (systolic)
Blood pressure (diastolic)
Weight
Temperature
Coronary Disease Risk Prediction Score
Smoking
Exercise

[0047] In another aspect of this invention, the therapeutic index is calculated using information derived from the patient's biological sample and patient information that is non-drug related. For example, in an ambulatory setting, information relating to concentration of drug, metabolite and other biological markers are measured in blood. These are termed the "device input". This input that is measured by a device used by the patient is then transmitted to a computer server hosted by the company. The patient also has the opportunity to directly input many non-drug related personal parameters. This is termed the "patient input" and relates to the patient's personal information such as height, weight,

gender, daily exercise status, food intake, etc. As can be imagined, the patient input could also be provided by the patient's healthcare provider. An example of a patient input parameter and the input means is shown in FIG. 6.

[0048] At the server level, the device input and patient input are used to compute the therapeutic index. As described earlier, a reference therapeutic index for the patient is already known using retrospective analysis of the data contained in the database. In formulating the therapeutic index using multiple regression analysis, the parameters such as those shown in Equation 1 are used. The same parameters are then used with the device input and patient input to compute the therapeutic index. Comparing the TI to the TI_{ref}, it is possible to determine the efficacy of the therapy. If the TI falls within a pre-determined range of TI_{ref}, then the treatment is considered to be efficacious. Values below that range indicate that the treatment is ineffective and values higher then the range are considered to be undesirable and could lead to adverse events.

[0049] Another example shows the implementation of this invention for studying the efficacy of therapy in diseases where it is hard to make frequent measurements and the efficacy of the treatment is hard to quantify. One example of such a situation in determining the efficacy of drug therapy in children with autism. Frequent sampling and concomitant laboratory analysis is impractical in the case of children. Abnormalities in blood concentrations of certain metals are implicated in autism. Hence, following the blood concentration of certain metals, e.g., zinc, in autistic children might shed light on the efficacy of an intervention. However, it has been reported that lowered concentrations of, say, Zn due to a treatment does not imply that the therapy is working. It is an indicator, but not a definitive surrogate for determining therapeutic efficacy. Computing a therapeutic index and comparing it to a reference level, would be quite beneficial in such situations. This is

illustrated in FIG. 7 by simulating the concentration of various pertinent markers and their change due to a drug intervention in an autistic child.

[0050] The program involves monitoring subjects and matched control individuals over time for (1) toxic metals, (2) surrogate markers for metals (metallothionein, etc.), and (3) other biochemical markers. Subjects are those prone to, or afflicted with autism; controls are situation-matched people. It is not mandatory that there be a situation-matched control. The scenario assumes that during the study a significant "event" occurs. Events could be: movement into a more or less risky environment; initiation of therapy, etc. Subjects could be frequently monitored by for several parameters (device input) using the ambulatory system described earlier. Additional laboratory assays that are not determinable in the ambulatory system could be performed at a lower frequency using laboratory assays. Additional data such as patient information, local environment, use of drugs, diet, etc. would be logged (patient input). Of specific interest to this scenario is information such as exposure to lead, mercury etc.

[0051] The time course shown in FIG. 7 envisages an event (initiation of therapy) at 33 days. The subject who is exhibiting abnormal levels of CP and MT, gradually reverts to normal levels of markers. The therapeutic index (TI) captures the risk or safety level of the subject based on all information. The study will define the best inputs to determine TI.

[0052] As described above, therapeutic index can be used for determining the efficacy of drug treatment. A similar approach is also well suited for determining the efficacy of drugs during clinical trials. Additionally, this approach could be beneficially used to identify sub-groups of patients who respond well or poorly to a given treatment regimen. The ability to segregate responders from non-responders is an extremely valuable tool. The concept of using TI can be used not only during a therapeutic regimen, but for performing diagnostic

tests to determine, for example, whether or not a patient is in need of a biopsy after a complete examination of prostate specific markers.

[0053] While the invention has been disclosed with reference to certain embodiments, it will be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the scope of the invention. In addition, many modifications may be made to adapt to a particular situation or material to the teachings of the invention without departing from its scope.

Abstract of the Disclosure

A method of computing a therapeutic index that could predict adverse drug reactions is disclosed. Adverse drug reactions could be due to unanticipated interactions between different drugs that a patient is taking, or the patient's unique negative reaction to a particular drug. By computing a reference therapeutic index by retrospective analysis of a patient population and by calculating the therapeutic index of an individual patent this invention is able to predict and avoid adverse drug reactions in individuals. Additionally, efficacy of drug or other therapeutic interventions can also be determined by computing a therapeutic index for such interventions.

FIG. 1: An illustration of how a reference therapeutic index would be computed.

Subject	Candidate output parameter					Input parameter					
	OP1	OP2	OP3	. .	OPn	IP1	IP2	IP3	. .	.	IPn
1											
2											
3											
.											
.											
.											
N											

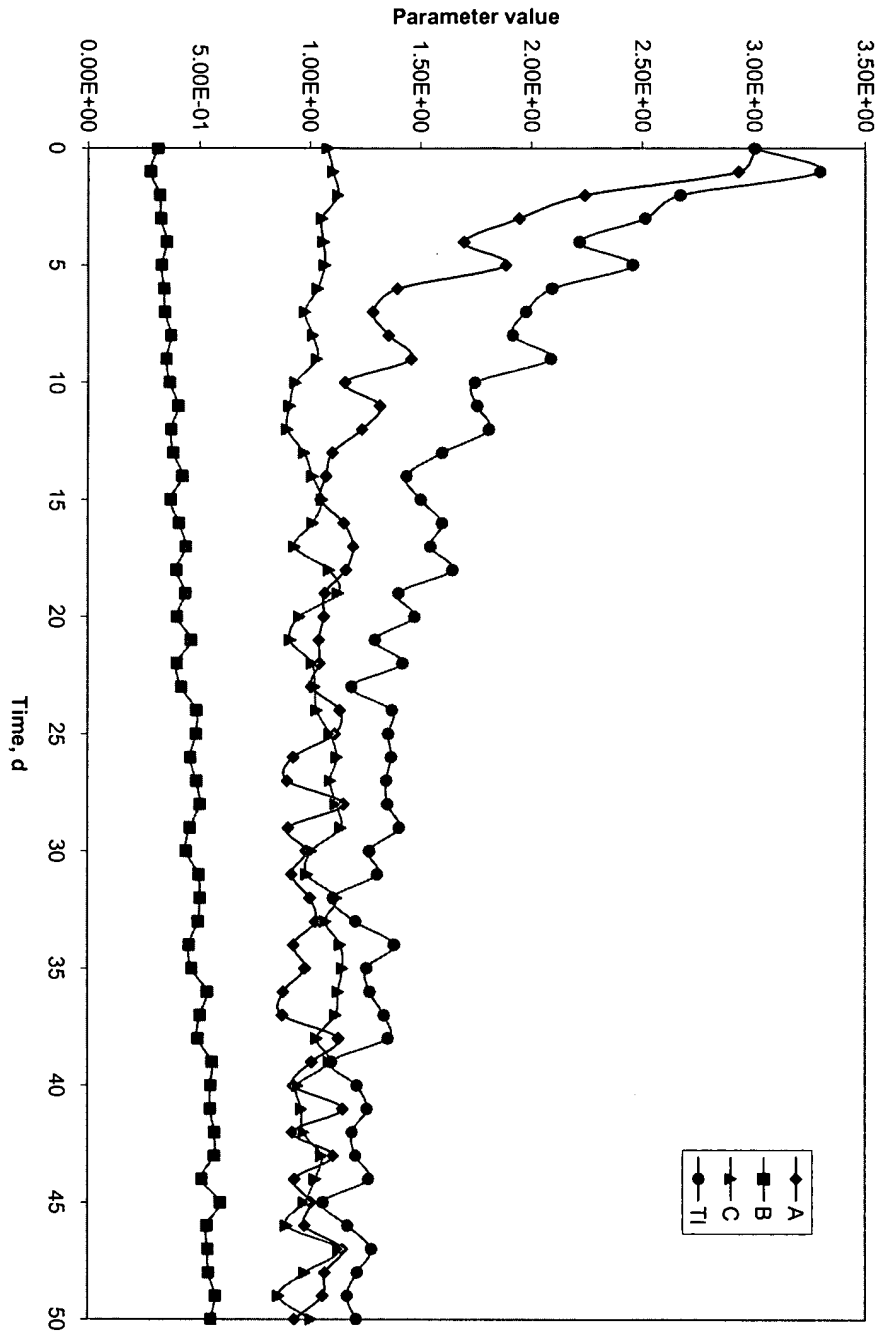

FIG. 2: Computing the Therapeutic Index (TI)

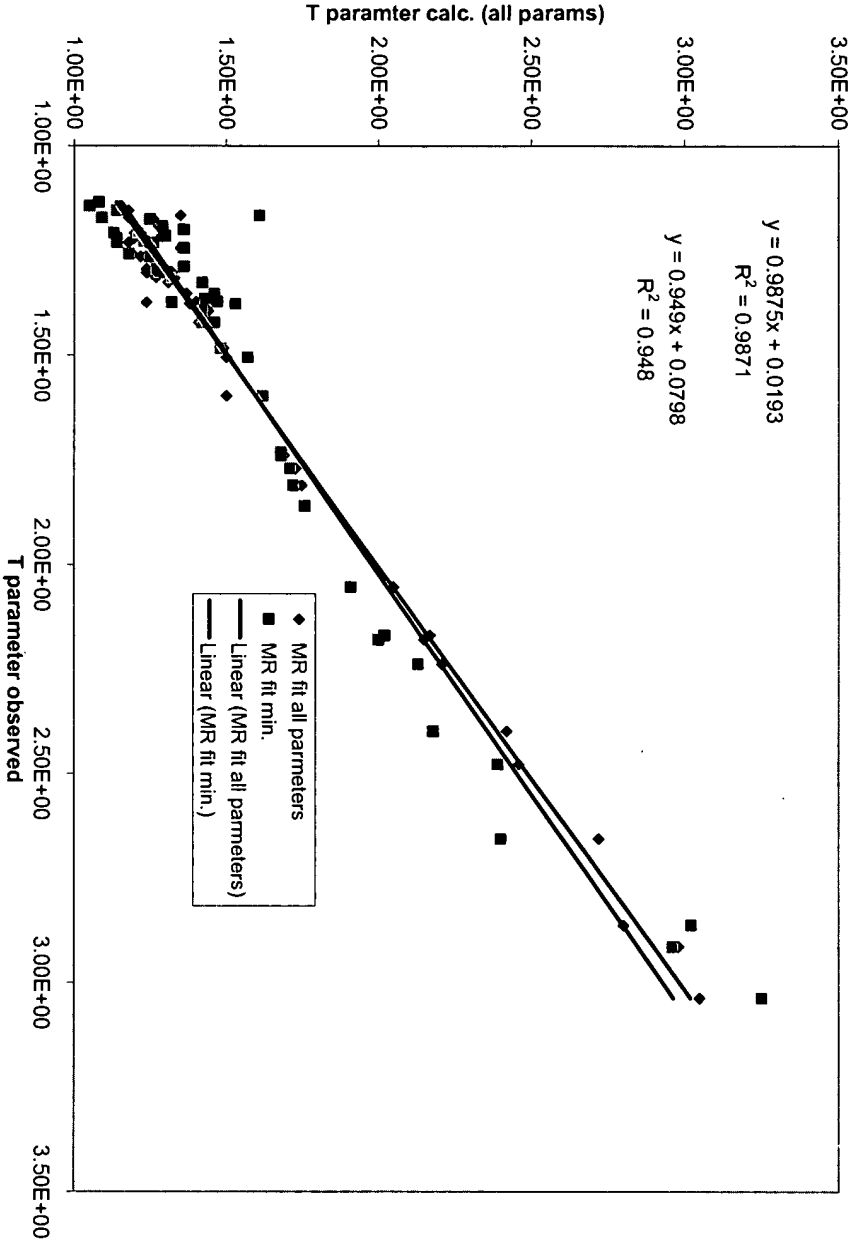

FIG. 3: Multiple Regression Analysis of the Computed TI

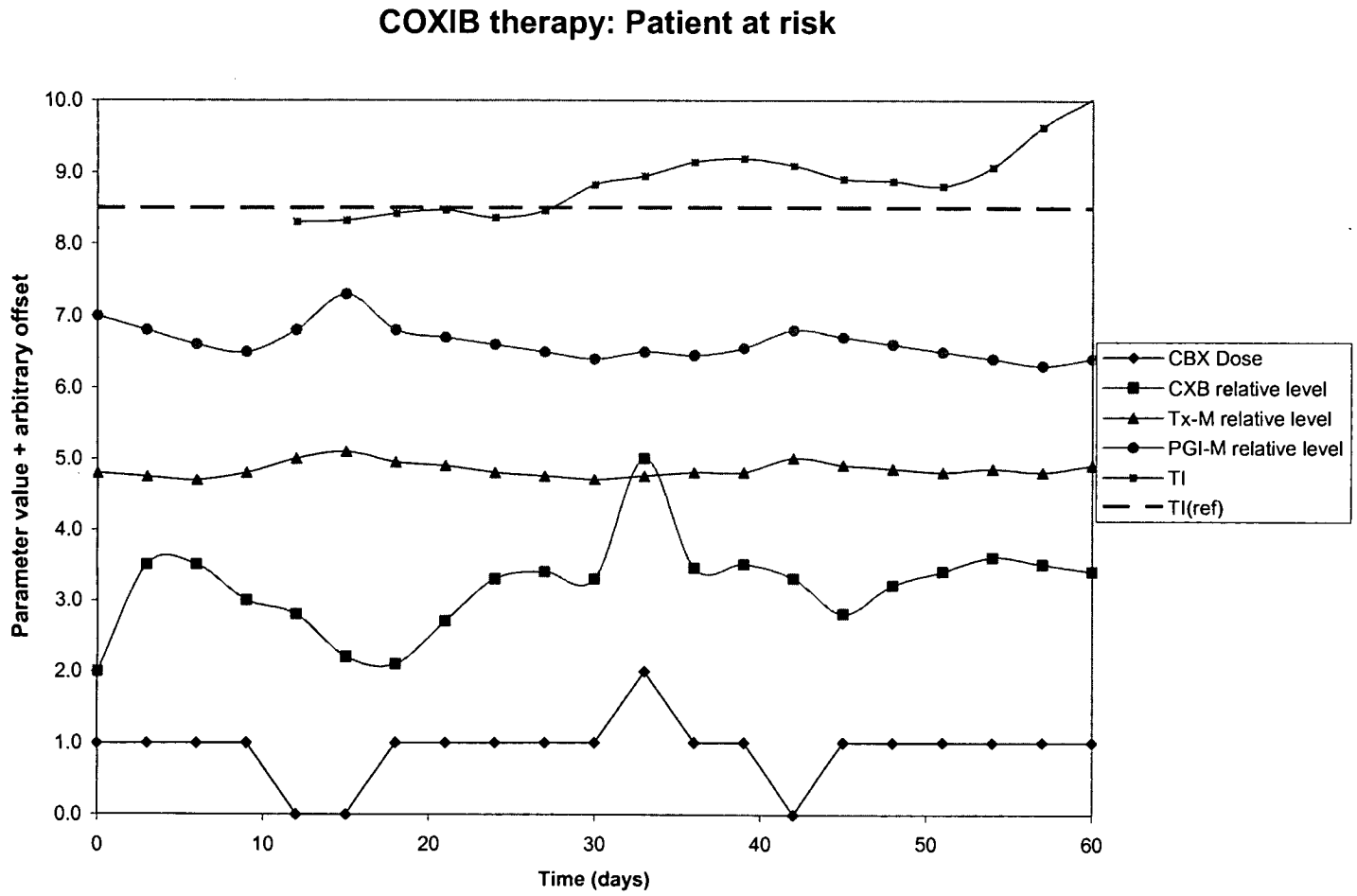

FIG. 4: Illustration of the relationship between measured drug, analyte and biomarker concentration and therapeutic index.

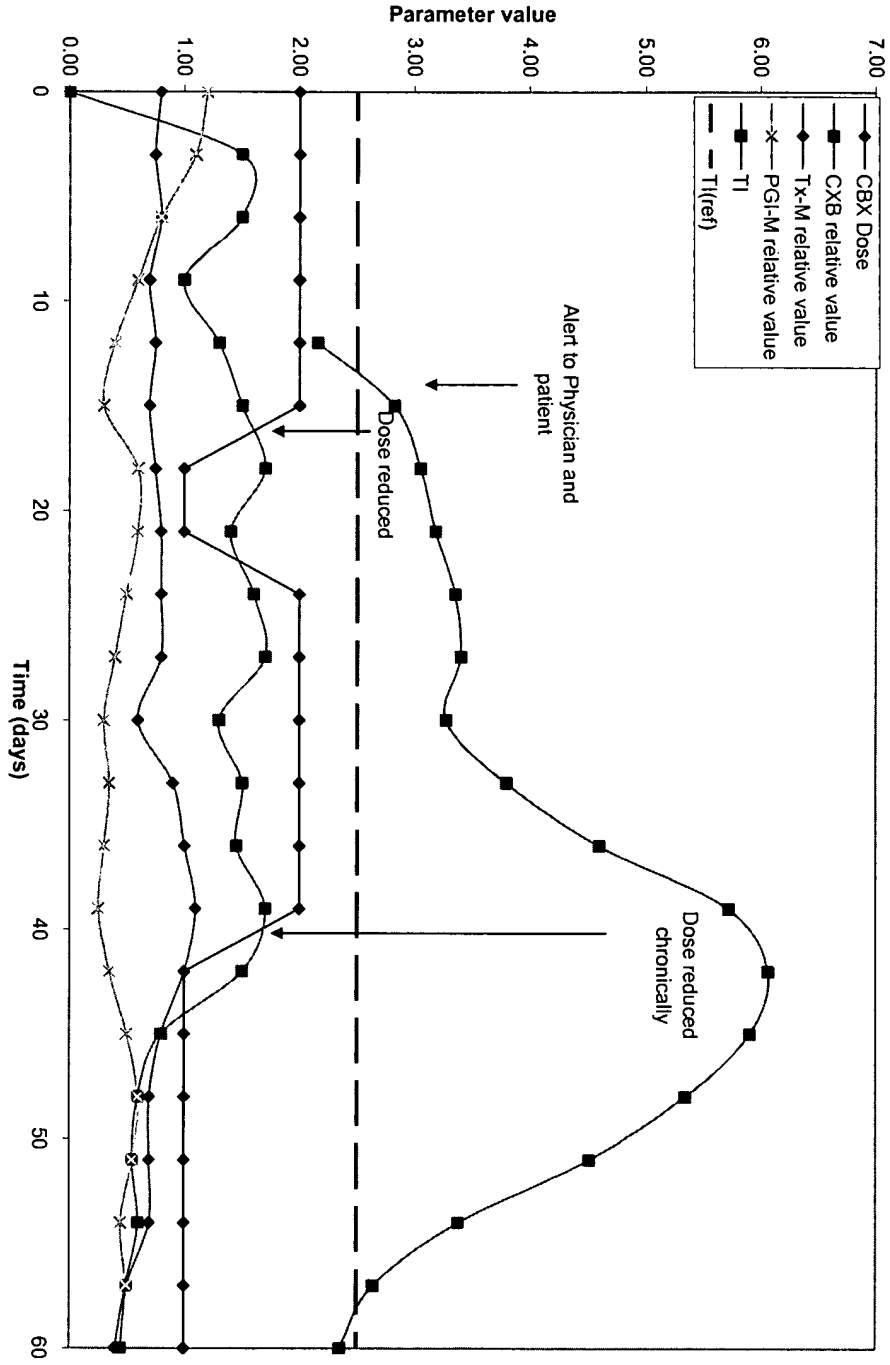

FIG. 5: Illustration of the application of this invention to minimize ADRs.

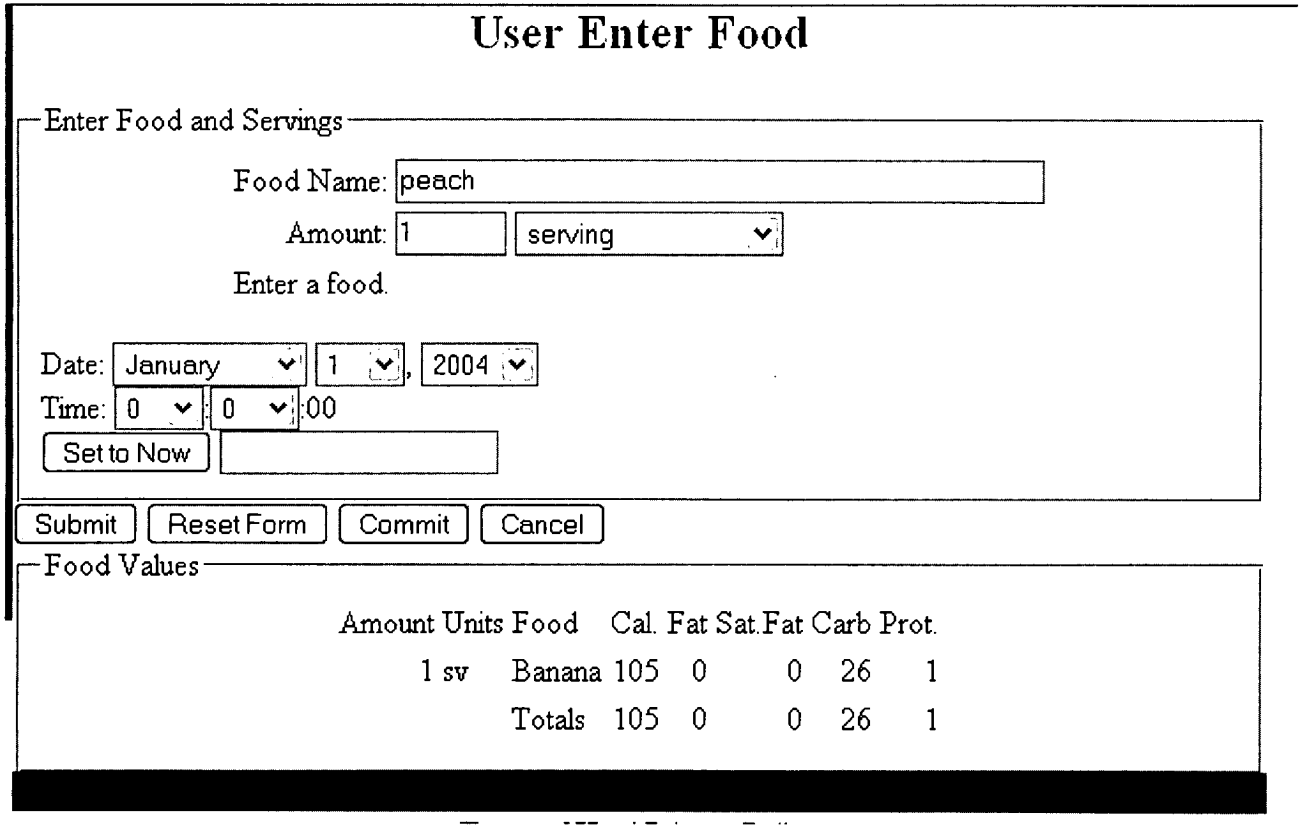

FIG. 6: Patient input values

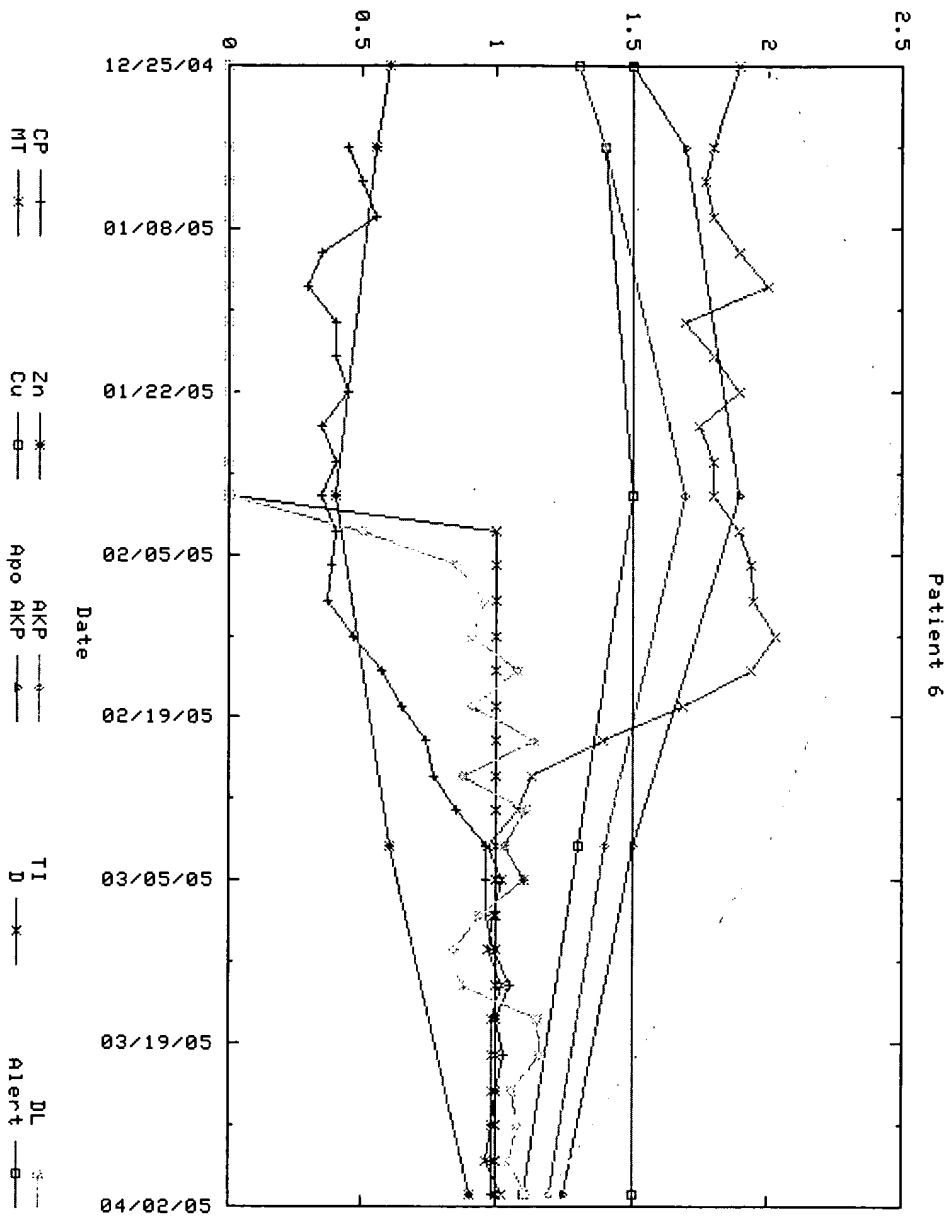

FIG. 7: Use of TI to follow treatment progression in an autism patient

PATENT APPLICATION SERIAL NO _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

09/19/2005 LWONDIM1 00000094 500417 60717192
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PTO-1556
(5/87)

U.S. Government Printing Office: 2002 — 466-247/69033

EXHIBIT D

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Express Mail Label No.				Docket Number	035738-0020
INVENTOR(s)/APPLICANT					
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Additional inventors are being named on the separately numbered sheets attached hereto.

TITLE OF THE INVENTION (500 characters max)

CONFIGURATIONS FOR IMPROVING PERFORMANCE OF A MICROFLUIDICS BASED DISPOSABLE DIAGNOSTIC SYSTEM

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of pages* [15] ☒ Small Entity Statement
☒ Drawings *Number of sheets* [9] ☐ Other (specify): _____

Application Size Fee: If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CAR 1.16(s).

METHOD OF PAYMENT OF APPLICATION SIZE FEE FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27. TOTAL FEE ($)

☐ A check or money order is enclosed to cover the filing fee and application size fee (if applicable). $100.00

☒ The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number: 500417 .

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

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35738-020

CONFIGURATIONS FOR IMPROVING PERFORMANCE OF A MICROFLUIDICS BASED DISPOSABLE DIAGNOSTIC SYSTEM

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1. Field of the Invention

[0001] This invention relates generally to loading, storing and dispensing liquids in a cartridge-based assay, particularly those based on a microfluidics system.

[0002] 2. Description of the Related Art

[0003] Point-of-Care (POC) testing systems and cartridges are becoming more common, as improvements in fabrication technologies, such as MEMS technology, enable reliable and inexpensive microfluidic based cartridges. Generally, such systems use microvalves, micropumps, microneedles, etc. for the proper flow of fluids through the system. A typical system will contain a reservoir for reagents, a mixing chamber, an analytical chamber and waste chambers. Fluids have to be moved from one chamber to another. Various publications describe the challenges associated with efficiently moving the fluids and mixing the reagents with the sample to be analyzed, problems in efficiently washing to remove unbound reagents from a measurement area, etc. One of the common challenges is washing the unbound conjugates after the incubation period. The most challenging part in washing the unbound conjugates is getting rid of those that are stuck on the edges of the assay wells. For example, US Patent No. 5,600,993 provides a good summary of such problems.

[0004] Various approaches have been described for enabling the fluid movement—electrical, osmotic, capillary, etc. For example, US Patent 6,440,725 describes different fluid motive sources for moving liquids through the chambers. One example uses a fluid that is inside a sealed pouch and the fluid is converted to gas by an electrical current. This action pressurizes and expands the fluid pouch. This sealed pumping pouch, or e-pump, is positioned against a reagent pouch and forces the contents of the reagent pouch into the fluidic circuit as the pumping pouch expands. The '725 patent also describes various other fluid motive sources such as

pressure or vacuum source, or using a solenoid or stepper motor to provide a force to press against a reagent pouch, etc.

[0005] Published US Patent Application No. 2005/0130292 A1 describes the use of mechanical energy to move fluids around in a cartridge. In this application, the inventors describe minimal or no external power to force the fluid through the various chambers. A sample is loaded on to a biochip and this biochip is inserted into a custom designed socket. The work done in inserting the socket is converted to the energy required for the microfluidic flow. For the subsequent steps of directing the sample to the desired chamber, mixing it and assaying it are, according to the inventors, accomplished with minimal power consumption. Such a device has many valves and pumps, even if the pumps are not driven by external electrical energy, which would not meet the goal of having a very small size for a disposable system.

[0006] Generally, reagents are kept in a dry state, to improve shelf-like and the like, in a POC system. Various buffers and the like are stored separately until the time when an assay has to be performed. The reagents are hydrated at that time to perform the intended analysis. It is a common problem for the dry reagents to become wet before the intended analysis time. The buffers may seep out of their holding areas and mix with the reagents. It will be beneficial to keep the dry reagents in a dry state until the assay is initiated.

[0007] Cartridge based POC systems handle small volumes of fluids. Nanoliter or even picoliter amounts of fluids are forced to flow around various fluid paths. Either during the sample introduction or the venting process, there is a significant chance for a bubble being introduced into the microfluidics system. Such a bubble could have serious negative consequences with respect to the assay.

[0008] Another common problem in microfluidics based system for assays is the problem of optical cross-talk. When assays with different luminescent intensities are run in adjacent reaction wells or chambers, photons (representing the signal generated) could travel from one

well to others. This optical cross-talk between wells will compromise the accuracy of measurements. The photons can travel through construction materials of the wells and through the fluidic channels that connect the wells. This problem is worsened, if the incubation time of assay gets longer. Hence, it will be beneficial to have appropriately designed fluidic channels and correctly chosen chamber material for eliminating optical cross-talk.

SUMMARY OF THE INVENTION

[0009] Various embodiments are provided to overcome some of the commonly encountered problems in cartridge based POC systems that employ microfluidics. One aspect of this invention is to provide means for long term stability of liquid reagents stored in reagent tanks in a cartridge system, provide barriers to isolate the dry reagents from the liquids present in the cartridge and keep the reagents dry during storage.

[0010] A microfluidics based cartridge device comprising reagent chambers containing one or more reagents, one or more mixing chambers, one or more reaction chambers and one or more waste chambers, wherein all the chambers are interconnected by a fluid flow path, and the reagents are initially retained in the reagent chambers by a burstable foil and are subsequently released from the reagent chambers by mechanical rupturing of the burstable foil.

[0011] A microfluidics based cartridge device comprising flexible reagent chambers containing one or more reagents, one or more mixing chambers, one or more reaction chambers and one or more waste chambers, wherein all the chambers are interconnected by a fluid flow path, and the reagents are forced from the reagent chambers by compressing the flexible reagent chambers.

[0012] A disposable cartridge for use in a POC system comprising a cartridge base with a microfluidic circuit and a fluid reservoir that is mated to the cartridge base, where the fluid reservoir has a top layer with pouches that hold the fluids and a bottom layer with a plurality of channels and the bottom layer is adhered to the cartridge base such that the channels of the fluid reservoir line up with the microfluidic circuit. Furthermore, the channels remain sealed until appropriate pressure is applied to unseal those channels. The applied pressure unseals or breaks the channels and enables the fluid to flow through the microfluidic circuit. Pressure could be applied using a pinch roller.

[0013] Another aspect of this invention is to prevent gas bubbles from interfering with assays in a microfluidics based cartridge system. A microfluidics cartridge based assay system comprising

a microfluidic circuit containing one or more reaction wells and a filter well that is fluidically connected to the reaction wells, wherein fluid flows in the microfluidic circuit first through the filter well and then through the reaction wells and the filter well contains a filtering medium such that any gas bubble in the fluid is trapped in the filter well.

[0014] Another aspect of this invention minimizes optical cross talk between reaction wells. Such cross talk compromises the accuracy of the signal measurements, as photons could travel from one well to another in a conventional design of the microfluidics circuit. This invention takes advantage of the fact that photons do not travel along a bend or a curve. Hence a system comprising a microfluidic circuit and or more reaction chambers and means for measuring the signals emanating from those chambers, wherein the reaction chambers are fluidically connected to each other, and such fluidic connections have bends or curves that do not allow photons to pass through. The walls of these chambers can be made of white or opaque materials so that the photons (signals) could be contained in the chambers.

[0015] In yet another embodiment of this invention, a method of minimizing measurement errors in microfluidics based cartridge system where conjugates stuck to the well of a reaction chamber contribute to those errors. One solution to avoid this problem is make the reaction chamber big enough that the conjugates stuck on edge will not contribute significantly to the reaction at the center of the well.

[0016] Other aspects of the invention include methods corresponding to the devices and systems described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The invention has other advantages and features which will be more readily apparent from the following detailed description of the invention and the appended claims, when taken in conjunction with the accompanying drawings, in which:

[0018] FIG. 1 is a schematic of a microfluidicss based cartridge for analyte assay.

[0019] FIG. 2 shows the fluid reservoir assembly and the various channels for filling and dispensing fluids.

[0020] FIG. 3 is a top, cross-sectional and bottom view showing the different layers of the fluid reservoir.

[0021] FIG. 4 shows a cross-section of the fluid reservoir assembly when mated to the cartridge.

[0022] FIG. 4A is an illustration of how compression dispenses the fluid from the reservoir into the wells in the cartridge.

[0023] FIGs. 5 and 5A illustrate another embodiment for preventing leaks and keeping the dry reagents isolated from the liquids in the cartridge.

[0024] FIG. 6 illustrates the inventive embodiment for minimizing optical cross talk in microfluidics based cartridge system.

[0025] FIG. 7 is a schematic of the microfluidic circuit for a bubble trapper.

[0026] FIGs. 8 and 8A illustrate the design for minimizing conjugates stuck on the edge or reaction wells.

[0027] FIG. 9 illustrates the use of a liquid absorbent to eliminate unintended leaks into the reader.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0028] Although the detailed description contains many specifics, these should not be construed as limiting the scope of the invention but merely as illustrating different examples and aspects of the invention. It should be appreciated that the scope of the invention includes other embodiments not discussed in detail above. Various other modifications, changes and variations which will be apparent to those skilled in the art may be made in the arrangement, operation and details of the method and apparatus of the present invention disclosed herein without departing from the spirit and scope of the invention as described here.

[0029] FIGs.1 and 1A illustrate of a microfluidic based cartridge system that can be used for determining analyte concentrations in biological samples. As shown in FIG. 1, cartridge **10** is made up of three layers—a cartridge base **11**, a reagent or reservoir layer **100** adhering to the base **11**, and a fluidic circuit **1000** above the reservoir layer **100**. The cartridge works in combination with a reader, as disclosed in co-pending U.S. Serial Number 60/678,801. FIG. 1A shows the top view of the cartridge **10** with all the components of the reservoir layer and the fluidic circuit. Generally, the cartridge contains a sample inlet means. This could be a hollow needle that is integrated in the cartridge, a strip or the like that could receive the blood sample that may have been collected using a lancet, etc. If necessary, the blood sample is processed by first diluting in a dilution chamber **1010**, and then filtering and separating the plasma in a filtration chamber **1020**. A bank of reagent chambers **1200** are present in the reservoir or reagent layer **100** of the cartridge **10**. The reagent chambers can contain substrates, wash buffers, conjugates and the like. The reagent reservoirs are fluidically connected to the reaction wells **1300**. The reaction wells **1300** could contain antibodies that would bind to the analytes. As in a typical immunoassay, these reaction wells would provide a signal indicative of the concentration of the analyte of interest. This signal is read by the reader that is associated with the cartridge.

[0030] In terms of the operation of the cartridge **10**, as soon as the cartridge is inserted into the reader, the assay steps are initiated. The first step in the assay is a wash cycle where all the surfaces of the fluidic circuit are wetted using a wash buffer. Then, the sample containing the analyte is introduced at the sample inlet. The sample is diluted at **1010** and separated into plasma or other desired component at the filter station **1020**. The separated sample now flows through the reaction wells **1300**, which have antibodies loaded in them and ready to bind to the appropriate analyte. The plasma of sample fluid is then flushed out of the reaction wells. A solution containing conjugates could be used for this flushing. The conjugates with enzymes attached to them, competitively bind to the antibody to form the enzyme-antibody complex. The unbound conjugate is then washed out of the reaction wells. An appropriate substrate is passed through the reaction wells with the enzyme-antibody complex. This action generates a chemiluminesence signal. This signal intensity is inversely proportional to the analyte concentration and the signal is then read by the reader. The cartridge contains one or more waste tanks **1400** and a calibration circuit that also contains reaction wells loaded with appropriate antibodies. All the wash buffers and other reagents used in the various steps, including the calibration step, are collected in the wash tanks.

[0031] One of the significant challenges in cartridge based assay that relies on microfluidic circuitry is the ability to store liquid reagents in the cartridge over a long period. Because of this problem, microfluidic based cartridges usually have a short shelf life. Liquid reagents undergo evaporative loss and considering the amount of reagents that is stored in the cartridge—usually in the microliter range—a cartridge could be devoid of any liquid reagent upon long enough storage. It can be easily understood that this has serious financial ramifications, as unused cartridges have to be disposed off if not used within the specified, short, time span. Furthermore, such evaporation can also contaminate the dry reagents, if there are any, stored in different locations on the cartridge. Finally, a robust seal is necessary for preventing liquid leakage during shipping.

[0032] Inventive embodiments described here can overcome the above problem. One such embodiment is shown in Fig. 2 illustrating the reagent reservoir assembly and release of fluids from these reservoirs to accomplish the above goals. A section of the cartridge **10** that contains the reagent reservoir assembly **1200** is shown in FIG. 2. FIG. 3 shows the top, front and bottom view of the reservoir assembly. The top layer **210** contains a plurality of bubbles or pouches **22** and a bottom surface **211**. A bottom layer **220** has a top surface **221** and a bottom surface **222**. Surface **221** is bonded to **211** and bottom surface **222** is bonded to the cartridge base **11**. The bottom layer **220** has a plurality of channels **23** dispersed through the entire surface, where each channel traverses the top surface **221** and the bottom surface **222**. As shown in FIG. 2, the fluid in the pouch is contained within the pouch by pressure burstable seal **24** between the channel **23** and the pouch,. The burstable seal **24** is designed such that at a pre-determined pressure the seal bursts allowing the fluid in the pouch **22** to flow out into the fluidic circuit through channel **23**.

[0033] The pouches **22** are filled using the two fill channels—fluid fill channel **300** and vacuum draw channel **310**. The process of filling the fluids (substrate, wash buffer, conjugates, etc.), involves first removing all the air from the pouch. This is done by drawing a vacuum through the vacuum draw channel **310**. Once the vacuum is drawn, a permanent seal **315** is placed between fill channel **300** and vacuum draw channel **310**. Next, required reagents are dispensed into the pouch through the fill channel **300**. Then, a permanent seal **316** is placed between the pouch **22** and the fill channel **300**. This ensures that when the pouch is compressed, the fluid can flow only in one direction, towards the burstable seal **24**. If the compression imparts a pressure larger than the burst pressure of seal **24**, the seal bursts and the fluid flows into the channel **23**. Channel **23** is in fluid communication with channel **1310** in the cartridge, which is illustrated in cross-section in Fig. 4. The reagent fluid could flow to the appropriate wells based on the microfluidic circuit. As shown in Fig. 4A, upon initiating the assay, each pouch is sequentially compressed by a roller **50** to compress pouches **22** and rupture the seal **24**. This forces the fluid out of the pouch into the corresponding dispensing channel **23**, which is in fluid communication with the fluidic circuit.

[0034] Another embodiment shown in FIG. 5 illustrates an inventive solution that addresses the above problems of liquid evaporation and leakage and isolates the liquids in the cartridge from the dry reagents until the assay is initiated by the user. Cartridge **10** contains a reagent tank **1100** covered by a burstable foil **1020**. Stacked on top of the burstable foil **1020** is the microfluidic circuit **1000**. A tough, but elastomeric top cover **1040** acts as the top most layer of the cartridge **10**. The reader that is used to read the signal output from the cartridge **10** contains a valve actuation plate **1050**. Securely attached to the plate **1050** is a non-coring needle **1060** such that when the plate is lowered, the sharp edge of the needle contacts the elastomeric cover **1040**. The top cover could be made of flexible silicone material that would act as a moisture impermeable seal.

[0035] To initiate the assay by releasing the reagents from the tank, the following sequence is orchestrated. FIG. 5A shows how the above assembly works. When the cartridge **10** is inserted into the reader, a linear stage pulls the cartridge into the reader and lowers the valve manifold **1050** into the cartridge. The series of non-coring needles **1060** then flex the top cover **1040**, as it is made of strong, flexible elastomeric material. However, the easily rupturable foil **1020** then ruptures due to the stress induced by the flexing of **1040** thereby releasing the reagent into the the fluidic circuit (not shown in diagram).

[0036] The same valve manifold **1050** can have an asymmetric pin **1060** inserted for each corresponding reagent tank. When the pins are lowered on to the cartridge they press into the silicone top **1040**, through the top fluidic circuit **1030** and break the foil seal **1020**. The pins remain in place as no mechanism is in place to pull out. Fluid can now be pumped through the circuit. The pins are lifted out of the cartridge as the cartridge is removed from the reader.

[0037] Each reagent reservoir could contain approximately 50µl to 1ml of fluid. More commonly the reservoir would contain about 100µl of fluid. As could be understood, the number of pouches and the volume contained in each pouch would vary depending on the nature of the assay and the sample that needs to be analyzed.

[0038] Figs. 6A through 6D illustrate another aspect of this invention. Fig. 6A illustrates the configuration of channels between reaction wells in existing microfluidic based cartridges. Reaction well **1300** is fluidically connected to the next reaction well by a linear flow fluid path **1310**. When assays with different luminescent intensities are run in adjacent reaction wells, photons (signals that emanate from the reactions) could travel from one well to the adjacent wells, as reaction wells are usually made of materials that allow photons to travel through the fluidic channels that connect the wells. This optical cross talk will compromise the accuracy of the measurements. Figs. 6B and 6C illustrate different embodiments of this invention that solve the optical cross talk problem. Non-linear channels, such as those with bends (**1320 or 1340**) or curves (**1330**) in them, will not allow photons (light) to pass through. Hence, embodiments such as those shown in Figs. 6B through 6D would not allow signals from the adjacent wells to contaminate the signal from the well that the signal reader is reading from. Additionally, the walls of the reaction wells are constructed using optically opaque materials so that light will not escape the wells. The material choice could be choosing an appropriate material that is white, or materials that are opaque.

[0039] One of the common problems encountered in a microfluidic based assay system is the presence of air or gas bubbles. Once gas bubbles are trapped in fluidic reaction chambers that contain micro inlet and outlet channels (fluid paths), it is extremely difficult to remove them. Bubbles present anywhere in the fluidic circuit, particularly in the reaction wells seriously compromise the assay capabilities. A bubble may end up occupying part of all of the surface area of a reaction well. Consequently the reader may end up reading a muted signal or no signal at all. Fig. 7 illustrates an embodiment where the bubbles could be trapped in a filter before they reach the reaction wells. A bubble trapper **72** is positioned between a liquid or gas inlet and the reaction wells. The bubble trapper **72** is of such a geometry that the bubbles tend to migrate towards the edges of this surface and remain stuck at that service, thereby not entering into the reaction wells.

[0040] During an immunoassay in a microfluidics based cartridge system, after the appropriate incubation period of usually about 2 minutes, the unbound conjugates have to be washed away. Otherwise, both the unbound and bound conjugates will activate the substrate (e.g., alkaline phosphatase) resulting in an incorrect signal. Because the reaction well volumes are in the microliter to picoliter range, and there is not excess wash fluid available, it is usually difficult to get rid of the conjugates stuck on the edges of the reaction wells. One approach to solving this problem is to make the contribution from the edge effect be much smaller compared to the signal emanating from the entire well. Fig. 8 illustrates this concept using the prior art configuration. Reaction well **1300** contains reaction surface **1350** and edge surface **1380**. In the inventive design shown in Fig. 8A, a much larger edge surface **1380A** is shown in conjunction with the reaction surface **1350.** This allows the unbound conjugates to adhere to the edges and be distanced from the bound conjugates, which are concentrated in the reaction surface **1350**.

[0041] The reader that is used in conjunction with the microfluidics based cartridge must be kept clear of liquids for preventing contamination of the reader. Vacuum is commonly used to move the fluids in the mircofluidic circuit. If there is a leak, it is possible that some of the liquids (reagents, waste, etc.) **910** could be sucked out of the cartridge and contaminate the reader. One inventive solution is to trap the liquid by converting the leaked liquid into a gel or other solid or semi-solid form. A liquid absorbing material **920**, such as polymeric materials found in diapers, could be placed in the channel that leads to the outlet that can come into contact with the reader. One example of such a polymer is sodium polyacrylate. Such polymers can absorb fluids hundreds of times their weight. Hence, only minute quantities of such polymeric materials may be required to accomplish the goal of absorbing leaked fluids. In one embodiment, all ports can be designed with channels filled with the superabsorbent material. This will result in a cartridge that is free of leaks.

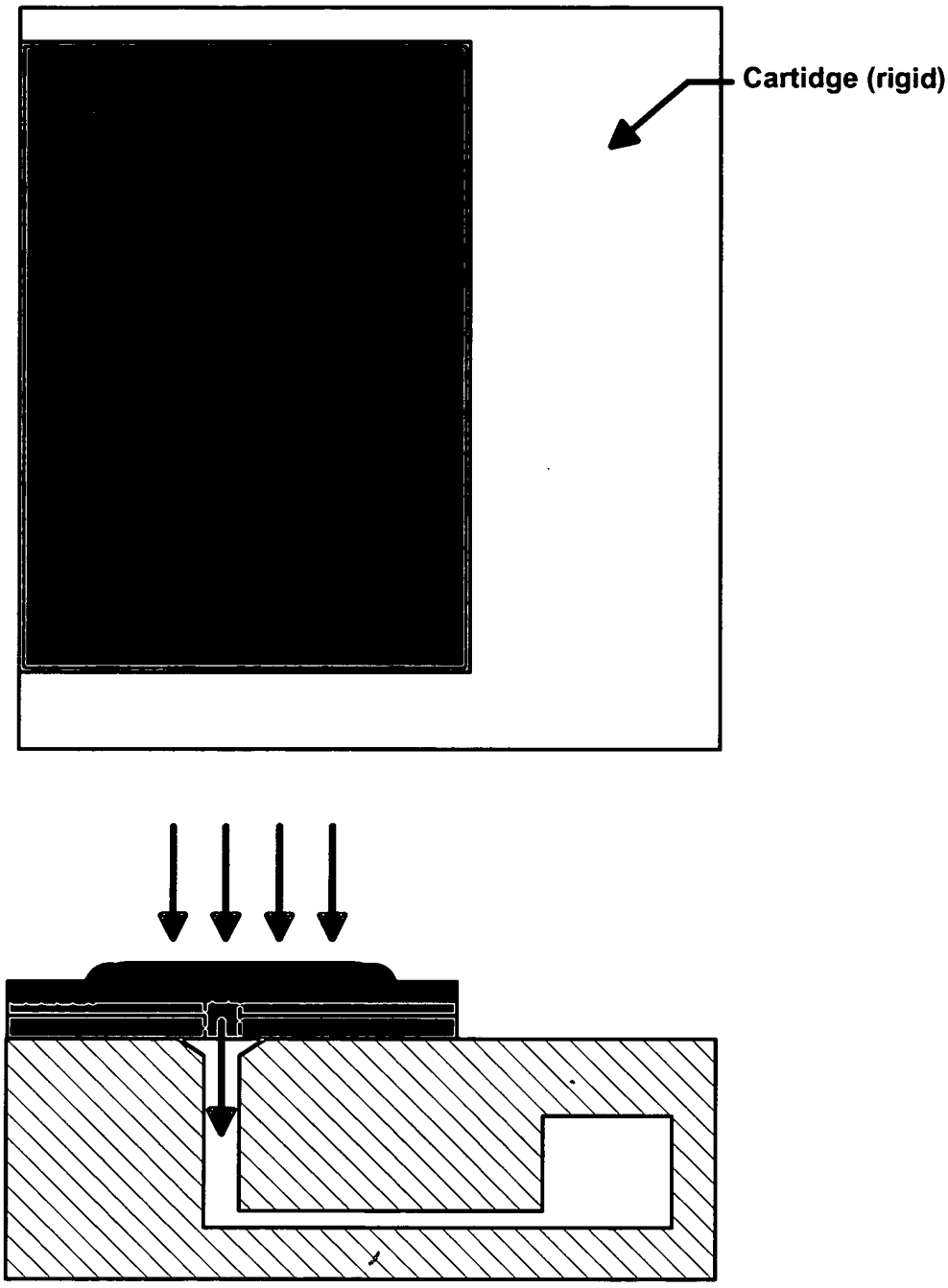
[0042] While the invention has been disclosed with reference to certain embodiments, it will be understood by those skilled in the art that various changes may be made and equivalents

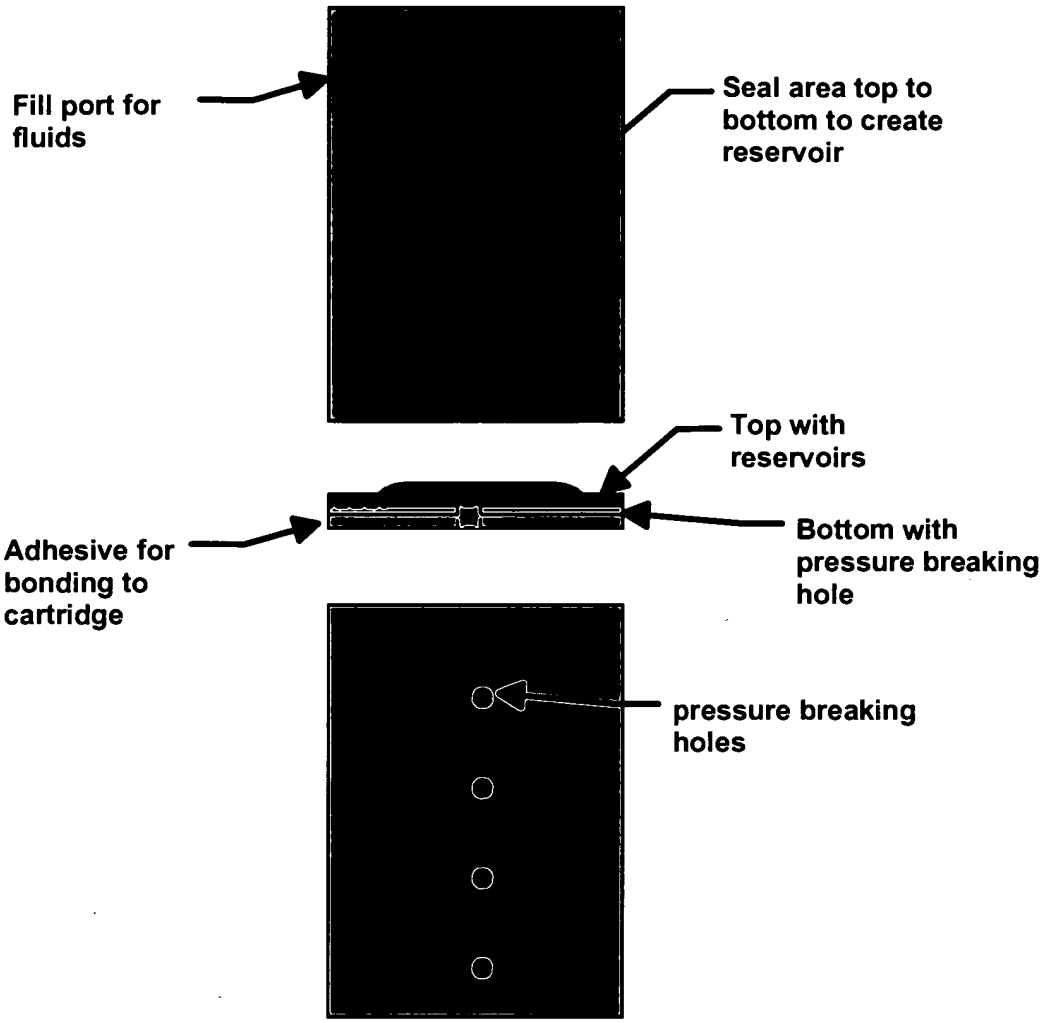
may be substituted without departing from the scope of the invention. In addition, many modifications may be made to adapt to a particular situation or material to the teachings of the invention without departing from its scope.

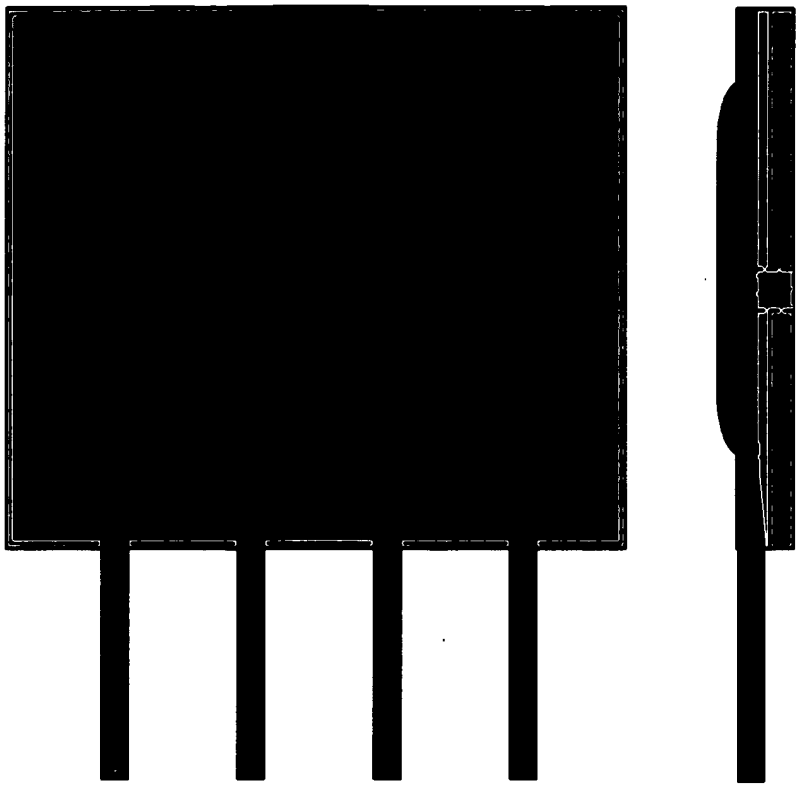
CONFIGURATIONS FOR IMPROVING PERFORMANCE OF A MICROFLUIDICS BASED DISPOSABLE DIAGNOSTIC SYSTEM

ABSTRACT OF THE DISCLOSURE

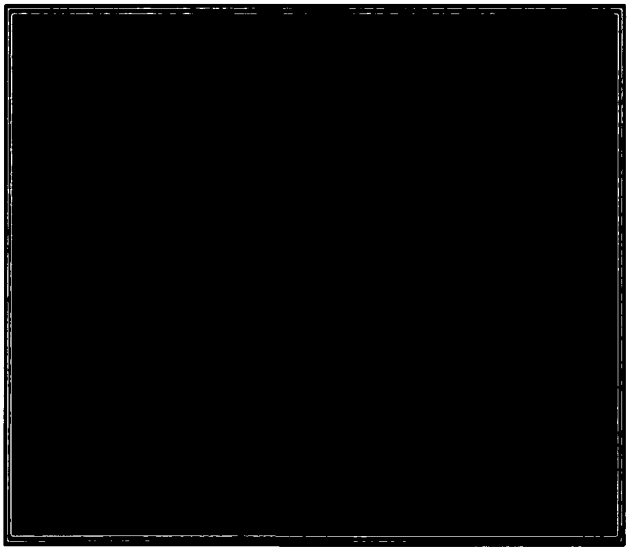
A microfluidics based cartridge for analyte assay is disclosed. Fluid flow is accomplished without the use of electrical energy and by using mechanical energy. This minimizes the size of the device and eliminates the need for expensive electronics, valves and pumps. Additionally, cartridge configurations are disclosed that isolate the dry reagents from the liquids in the cartridge and methods of increasing shelf life of the cartridges. Other aspects of the invention eliminates bubbles in the microfluidics circuit and minimizes optical cross talk between reaction wells.


Cartidge (rigid)


Fill port for fluids
Seal area top to bottom to create reservoir
Top with reservoirs
Adhesive for bonding to cartridge
Bottom with pressure breaking hole
pressure breaking holes

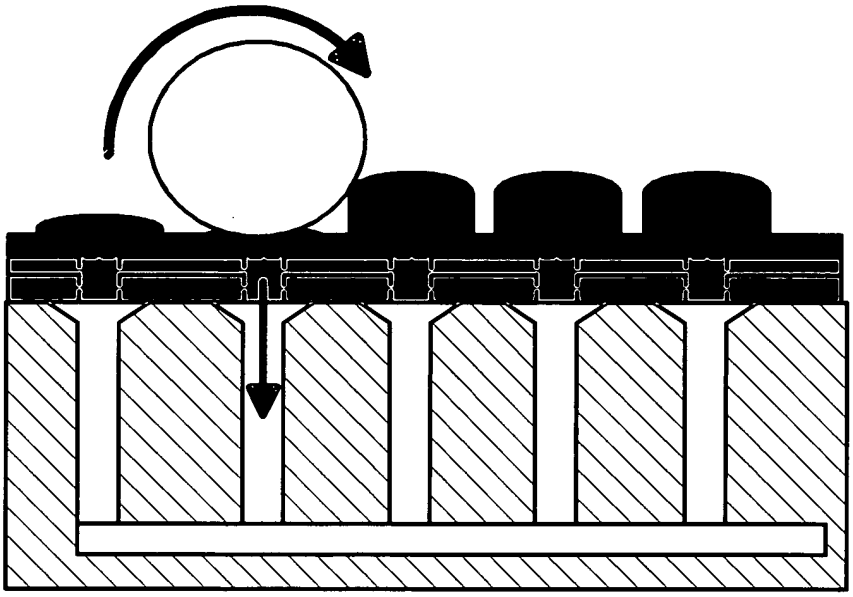


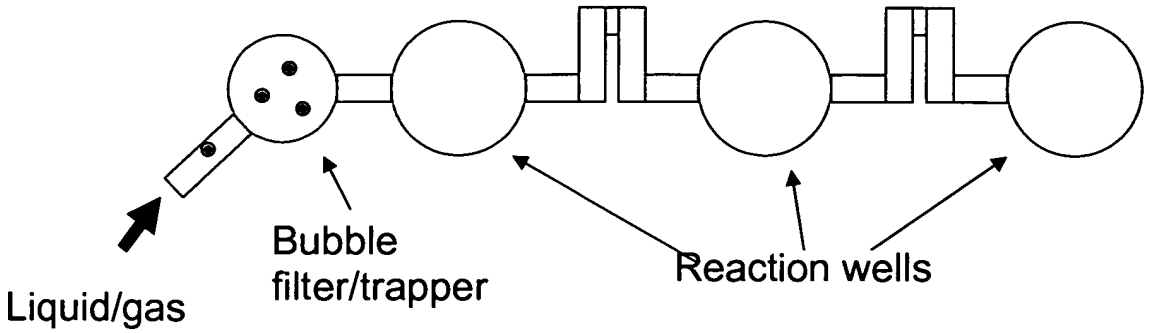
Fluids intoduced into reservior

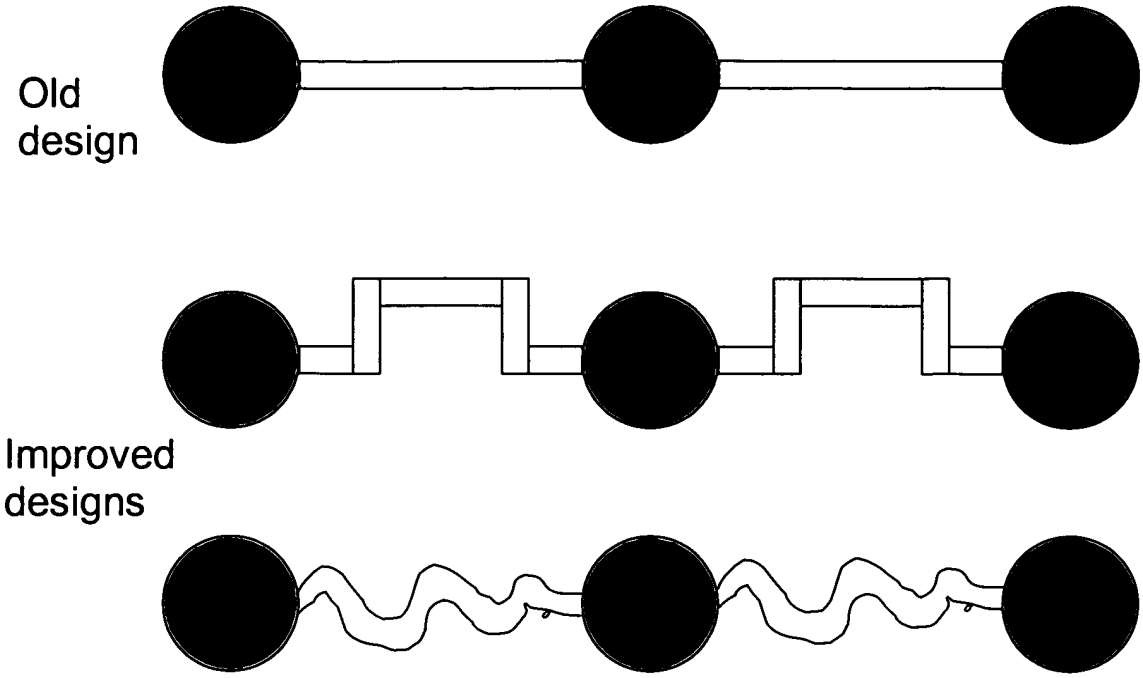


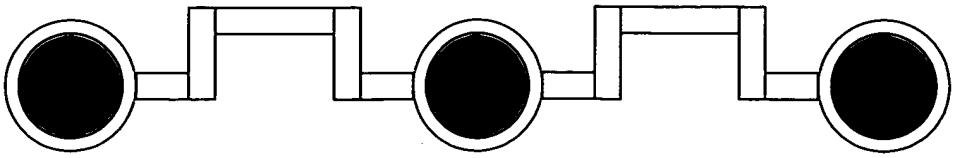
Needles retracted and fill ports sealed

Reservoir actuation

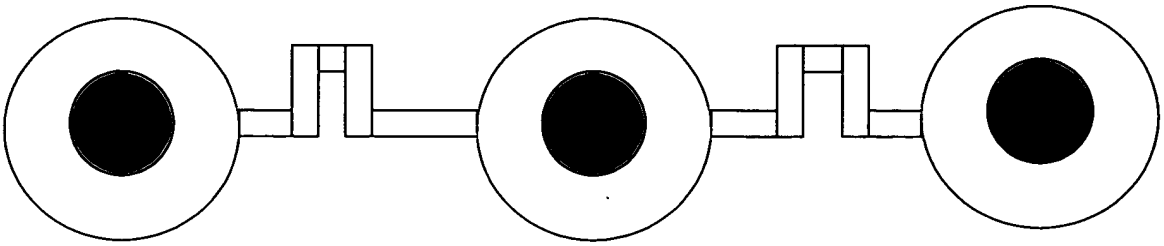



Liquid/gas
Bubble
filter/trapper
Reaction wells

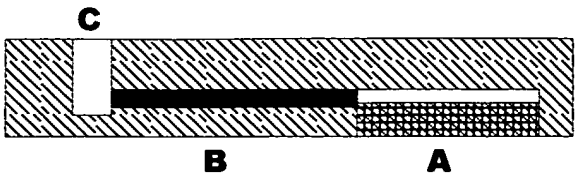

Old
design
Improved
designs



Old design, conjugates stuck on edge will contribute to overall reaction



Improved designs, conjugates stuck on edge will contribute much less to overall reaction


C
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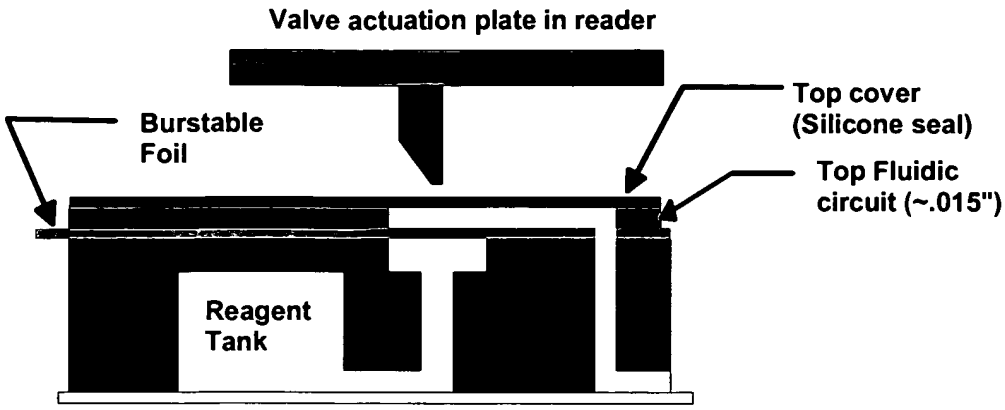
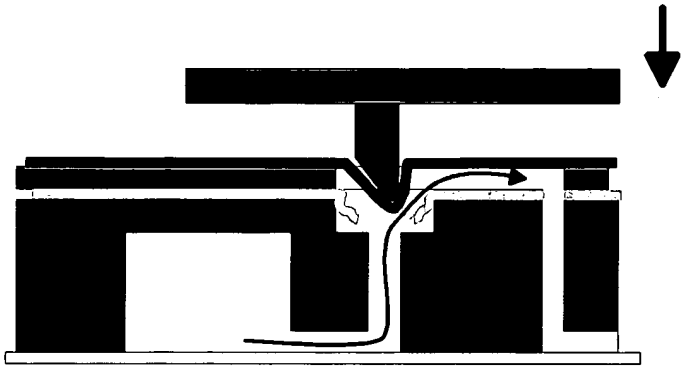


Cartridge with sealed wells



Cartridge with seals burst

PATENT APPLICATION SERIAL NO _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Richard C. Joseph M	FUISZ FUISZ	McLean VA Washington, D.C.

☐ *Additional inventors are being named on the separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Method for Programming a Bodily fluid Analyzer, Bodily Fluid Analyzer, and System Including a Bodily Fluid Analyzer

Direct all correspondence to: CORRESPONDENCE ADDRESS

☒ Customer Number 20457

OR

☐ Firm *or* Individual Name					
Address					
Address					
City		State		Zip	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS (*check all that apply*)

☒ Specification *Number of Pages 14*
☐ CD(s), Number
☐ Drawing(s) *Number of Sheets*
☐ Other (specify)
☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 01-2135.

☒ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount$ **$100.00**

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No
☐ Yes, the name of the U.S. Government agency and the Government contract number are:

Date 04/24/2006

Respectfully submitted,

SIGNATURE

REGISTRATION NO. 32,087 *(if appropriate)*
Docket Number: 847.46141L00

TYPED or PRINTED NAME Alan E. Schiavelli

TELEPHONE 703-312-6600

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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847.46141L00

TITLE:

Method for Programming a Bodily fluid Analyzer, Bodily Fluid Analyzer, and System Including a Bodily Fluid Analyzer

BACKGROUND OF THE INVENTION:

Devices for measuring bodily fluid analytes, especially blood analytes such as glucose, are known. Recently, attempts have been made to provide such devices for home use.

Devices for measuring bodily fluid analytes are described in United States Patent Nos. 5,980,830, 6,146,510, 6,259,562, 6,302,855, 6,845,327 and 7,027,849 and United States Patent Application Publication Nos. 2005/0100937A1 and 2006/0062852A1.

United States Patent Publication No. 2004/0106859 A1 to Say et al discloses an analyte monitoring device and methods of use, and discloses that the device may include an optional alarm system that warns the patient of a potentially detrimental condition of the analyte. The alarm system is triggered when the data from the processing circuit reaches or exceeds a threshold value. The analyte monitor device may be configured so that the threshold levels may be programmable by the patient and/or a medical professional. In addition, it is disclosed that, in some embodiments of the invention, the device may include a transmitter configured to transmit data to another receiver/display unit or some other receiver. As an example, it is disclosed that a receiver/display unit may transmit data to a computer in the patient's home or at a doctor's office.

Moreover, the transmitter or a separate transmitter may direct a transmission to another unit or to a telephone or other communications device that alerts a doctor or other individual when an alarm is activated and/or if, after a predetermined time period, an activated alarm has not been deactivated, suggesting that the patient may require assistance. In some embodiments, the receiver/display unit is capable of one-way or two-way paging and/or is coupled to a telephone line to send and/or receive messages from another, such as a health professional monitoring the patient.

A biomonitoring and informatics system is under development by Theranos, Inc. and is described on the company website as follows.

> Theranos is preparing to launch the first personalized biomonitoring and informatics system to monitor the effects of prescription medicines. Theranos devices enable healthcare professionals and pharmaceutical companies to track patients' individual responses to prescribed medicines, painlessly and in real time, throughout the course of treatment.
>
> Based on a proprietary process, the Theranos handheld monitors simultaneously detect changes in the levels of biochemical markers directly induced by the drug, then wirelessly communicate results to medical personnel through a bioinformatics server.
>
> The Theranos platform consists of a Reader and disposable cartridges that analyze a specific prescription medicine. The Reader automatically transmits analysis data to the HIPAA-compliant Theranos database, which rigorously protects patient identity information while making available to healthcare professionals detailed, high-level and real-time information.
>
> The highly portable, easy-to-use Reader can be used with all Theranos disposable cartridges, meaning that individuals can use the same Reader to gather and transmit information on any number of targeted prescription medicines.

However, applicants have found that what is still needed is a device, especially a home use device, and method that can make programming of such a device simple and specific to a particular drug or course of treatment.

SUMMARY OF THE INVENTION:

The present invention relates to a method for programming a bodily fluid analyzer, including providing a data storage unit containing stored information concerning a particular drug being or to be taken by the patient or course of treatment for the patient; reading the stored information stored on the data storage unit into a data reader associated with a bodily fluid analyzer; setting at least one threshold value for at least one analyte to be sensed by the bodily fluid analyzer based on the information read by the data reader from the data storage unit, wherein the threshold value is associated with the particular drug being or to be taken by the patient or course of treatment for the patient, the threshold value being one beyond which the display will display an alert; sensing the analyte; and displaying an alert if the sensed analyte level is beyond the threshold value.

The present invention also relates to a bodily fluid analyzer including a sensor for sensing at least one bodily fluid analyte in a patient; a display for displaying processed information concerning the sensed analyte; a data reader unit for reading information from a data storage unit, the data storage unit containing stored information concerning a particular drug being or to be taken by the patient or course of treatment for the patient; and a processor for setting the at least one threshold value for at least one analyte to be sensed by the sensing unit based on the information read by the data reader from the data storage unit, for processing the information concerning the analyte and for sending the processed information to the display, wherein the threshold value is associated with the particular drug being or to be taken by the patient or course of treatment

for the patient, the threshold value being one beyond which the display will display an alert.

The present invention also relates to a system for monitoring a patient, including such a bodily fluid analyzer and a data storage unit that may be provided with a drug container.

The bodily fluid may be blood, urine, etc.

DETAILED DESCRIPTION OF THE INVENTION:

Devices for measuring bodily fluid analytes are described in United States Patent Nos. 5,980,830, 6,146,510, 6,259,562, 6,302,855, 6,845,327 and 7,027,849 and United States Patent Application Publication Nos. 2005/0100937A1 and 2006/0062852A1, the content of each of which (including the drawings thereof) is incorporated herein in its entirety. The bodily fluid analyzer of the present invention includes a sensor for sensing at least one analyte in a patient; a display for displaying processed information concerning the sensed analyte; and a processor for processing the information concerning the analyte and for sending the processed information to the display. The sensor, the display and this basic aspect of the processor (processing the information concerning the analyte and for sending the processed information to the display) can be of the type described in United States Patent Nos. 5,980,830, 6,146,510, 6,259,562, 6,302,855, 6,845,327 and 7,027,849 and United States Patent Application Publication Nos. 2005/0100937A1 and 2006/0062852A1.

The present invention modifies such devices by, *inter alia*, including a data reader unit for reading information from a data storage unit, the data storage unit

containing stored information concerning a particular drug being or to be taken by the patient or course of treatment for the patient; and a processor for setting the at least one threshold value for at least one analyte to be sensed by the sensing unit based on the information read by the data reader from the data storage unit, for processing the information concerning the analyte and for sending the processed information to the display, wherein the threshold value is associated with the particular drug being or to be taken by the patient or course of treatment for the patient, the threshold value being one beyond which the display will display an alert.

The data storage unit may be provided with a drug container or otherwise supplied by a caregiver to the patient. For example, the data storage unit can be a bar code and the data reader can be a bar code reader; the data storage unit can be a radio frequency identification tag and the data reader a radio frequency receiver; the data storage unit can be a magnetic stripe and the data reader a magnetic stripe reader. Thus, the patient can merely scan the bar code, move the radio frequency identification tag near the reader or swipe the magnetic stripe so that the data associated with the particular drug being or to be taken by the patient or course of treatment for the patient is read in a simple and foolproof manner.

The data stored on the data storage unit may be preset parameters to be monitored, parameters that are important for the particular drug or course of treatment. Alternatively, the data stored on the data storage unit may store parameters that are set by the prescribing physician specifically for that particular

patient or by the prescribing physician or drug company for a class of patients (e.g., elderly). Alternatively, if, e.g., if the data storage unit stores preset parameters, the device may contain an input unit (physically connected or remotely connected) so that the physician can narrow or widen the parameters to meet the special physiology of a particular patient. For example, the physician may well want a wider creatinine tolerance in an 80 year old than in a 20 year old. The processor could also calculate, e.g., creatinine clearance or other analyte limits from the patient's age, height, weight and measured serum creatinine.

For example, it is known that Captopril™ or Lisinopril™ can lead to elevated potassium (K) levels. Therefore, according to the present invention, the data storage unit may contain preset parameters regarding K levels to be monitored, e.g., the data storage unit may be used to program the device to set alarms when the K level is outside the range of 3.5-5.0 Meq/L. Alternatively, the data stored on the data storage unit may contain parameters that are set by the prescribing physician specifically for that particular patient or by the prescribing physician or drug company for a class of patients (e.g., elderly), e.g., the prescribing physician may want to store data to bump the preset K range up to 5.5 if the patient chronically ran high.

For example, it is known that Furosemide™ can lead to elevated creatinine or low K levels. The data storage unit may be used to program the device to set alarms when the K level is outside the range of 3.5-5.0 Meq/L or the creatinine 0.6-1.2 Mg/dL. However, if the patient's if the baseline creatinine is

1.6, the physician could change the creatinine range to 1.2 to 2.0. This can be done by having the data stored on the data storage unit set by the prescribing physician specifically for that particular patient or, alternatively, the device may contain an input unit (physically connected or remotely connected) so that the physician can narrow or widen the parameters to meet the special physiology of a particular patient.

For example, the data storage unit associated with a drug container containing Lipitor™ can set ALT> 150 iu/L as an upper limit (normal is 48). The data storage unit associated with a drug container containing Metformin™ can set creatine clearance <60 ml/min.

For example, it is known that a normal level of potassium is 3.5 - 5 meq/L; hypokalemia is defined when potassium level is below 3.5 meq/L. The normal level for sodium (Na) is 136 -145 meq/L; hyponatremia occurs when sodium level is below 130 meq/L. Prescription of a thiazide diuretic in primary care can be associated with high frequency of hyponatrimia and hypokalemia. The risk of hyponatremia, especially in the elderly, should be considered and monitored. Therefore, a drug container containing a thiazide diuretic would have a data storage unit on having data stored with preset parameters from which the processor can set at least one threshold value for K or Na, e.g., set alarms if the K level is below 3.5 meq/L or the Na level is below 130 meq/L.

If the analyzer is of the type that uses disposable cartridges, the data storage unit may contain information as to what a particular drug is and the processor processes this information to display it on the display so that the

patient does not put the wrong cartridge (i.e., a cartridge for the wrong drug) in the analyzer. The processor could also give an alert if the wrong cartridge is accidentally place in the analyzer.

The present invention enables the linkage of transmission to the analyzer Bios system. This may be done according to the present invention by the standard drug container having more information on it to communicate with the analyzer as to what is to be measured. The present invention has unexpectedly advantageous results as compared to known systems. For example, with respect to the analyzer described by Theranos, the analyzer is claimed to be module specific; however, if the patient were responsible for placing the proper module in the analyzer, this would be a compliance nightmare. The present invention will enable a huge advance in medical care by simplifying the home analysis of fluids for monitoring health as well as for the monitoring of a specific drug (e.g., a drug company clinical trial). The present invention will enable the pill container via a reader to give full instruction to the analyzer as to what the fluid is and what parameter is to be measured. This presupposes that future drug containers will contain this information, e.g., on a data storage unit, or that the care provider otherwise gives the information to the patient. As time gains, the system can be further advanced by the additional data contained on the container and read by the reader of wider or narrower or higher or lower values of a particular analyte for a particular patient.

While the present invention is particularly described with respect to blood analyzers, those skilled in the art would understand, from the foregoing

description, that the invention could be used with analyzers of other bodily fluids. The data storage unit may contain information the processor can use to determine the fluid to be analyzed.

The data storage unit may contain a universal analyzer code by which the physician can prescribe the code on a container or directly to a reader- not simply of the fluid analyzed and the specific analyte, but the range of normal as well. For example, a theoretical code BK1 or BK2 or BK3 will mean Blood-Potassium and range of normal schema 1 or 2 or 3.

In the future, the drug itself may contain the information read through its individual package or through a microchip in the dosage form itself.

We claim:

1. A method for programming a bodily fluid analyzer, comprising:

providing a data storage unit containing stored information concerning a particular drug being or to be taken by the patient or course of treatment for the patient;

reading the stored information stored on the data storage unit into a data reader associated with a bodily fluid analyzer;

setting at least one threshold value for at least one analyte to be sensed by the bodily fluid analyzer based on the information read by the data reader from the data storage unit, wherein the threshold value is associated with the particular drug being or to be taken by the patient or course of treatment for the patient, the threshold value being one beyond which the display will display an alert;

sensing the analyte; and

displaying an alert if the sensed analyte level is beyond the threshold value.

2. The method according to claim 1, wherein the data storage unit is provided with a drug container.

3. The method according to claim 2, wherein the data reader is a bar code reader and the data storage unit is a bar code.

4. The method according to claim 2, wherein the data reader is a radio frequency receiver and the data storage unit is a radio frequency identification tag.

5. The method according to claim 2, wherein the data reader is a magnetic stripe reader and the data storage unit is a magnetic stripe.

6. A bodily fluid analyzer comprising:

a sensor for sensing at least one analyte in a patient;

a display for displaying processed information concerning the sensed analyte;

a data reader unit for reading information from a data storage unit, the data storage unit containing stored information concerning a particular drug being or to be taken by the patient or course of treatment for the patient; and

a processor for setting the at least one threshold value for at least one analyte to be sensed by the sensing unit based on the information read by the data reader from the data storage unit, for processing the information concerning the analyte and for sending the processed information to the display, wherein the threshold value is associated with the particular drug being or to be taken by the patient or course of treatment for the patient, the threshold value being one beyond which the display will display an alert.

7. The bodily fluid analyzer according to claim 6, wherein the data reader is a bar code reader.

8. The bodily fluid analyzer according to claim 6, wherein the data reader is a radio frequency receiver.

9. The bodily fluid analyzer according to claim 6, wherein the data reader is a magnetic stripe reader.

10. The bodily fluid analyzer according to claim 6, wherein the display is provided proximate the sensor.

11. The bodily fluid analyzer according to claim 6, wherein the display is provided in an office of a health care professional caring for the patient.

12. A system for monitoring a patient, comprising the bodily fluid analyzer according to claim 6 and a data storage unit containing stored information concerning a particular drug being or to be taken by the patient or course of treatment for the patient.

13. The system according to claim 12, wherein the data storage unit is associated with a drug container

14. The system according to claim 12, wherein the data reader is a bar code reader and the data storage unit is a bar code.

15. The system according to claim 12, wherein the data reader is a radio frequency receiver and the data storage unit is a radio frequency identification tag.

16. The system according to claim 12, wherein the data reader is a magnetic stripe reader and the data storage unit is a magnetic stripe.

17. A method for using a bodily fluid analyzer using disposable cartridges, comprising:

providing a data storage unit associated with a drug container, the data storage unit containing stored information identifying a particular drug in the drug container;

reading the stored information stored on the data storage unit into a data reader associated with a bodily fluid analyzer;

displaying on a display of the analyzer the disposable cartridge, associated with the particular drug, to be inserted into the analyzer;

inserting a disposable cartridge into the analyzer; and

displaying an alert if the disposable cartridge inserted into the analyzer does not match the disposable cartridge associated with the particular drug.

ABSTRACT:

A bodily fluid analyzer and method for programming the same includes a sensor for sensing at least one analyte in a patient; a display for displaying processed information concerning the sensed analyte; a data reader unit for reading information from a data storage unit, the data storage unit containing stored information concerning a particular drug being or to be taken by the patient or course of treatment for the patient; and a processor for setting the at least one threshold value for at least one analyte to be sensed by the sensing unit based on the information read by the data reader from the data storage unit, for processing the information concerning the analyte and for sending the processed information to the display, wherein the threshold value is associated with the particular drug being or to be taken by the patient or course of treatment for the patient, the threshold value being one beyond which the display will display an alert. A system for monitoring a patient includes such a bodily fluid analyzer and the data storage unit.

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